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POLYAMINES FROM REACTION OF ORGANOTIN DIHALIDES AND 3-AMINO-1,2,4-TRIAZOLE (3-AT)- SYNTHESIS AND ABILITY TO INHIBIT HUMAN CANCER CELL LINES

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ABSTRACT

The formation of polyamines from reaction of 3-amino-1,2,4-triazole, 3-AT, with organotin dichlorides occurs rapidly employing the interfacial polycondensation reaction giving moderate yield and chain lengths. MALDI MS produces ion fragments to seven and nineteen units with good isotopic abundance matches. IR shows the absence of the internal ring NH consistent with the formation of Sn-N bands and inclusion of bands from both the organotin and 3-AT units. NMR results are also consistent with the proposed repeat unit. The polymers show good inhibition of a group of solid human cancer cell lines including breast, lung, prostrate, and pancreatic cancer and glioblastomas brain cancer cell lines. Thus, these polymers offer a wide range of ability to inhibit cancer cell lines.

KEYWORDS: 3-amino-1,2,4-triazole, 3-AT, organotin, brain cancer, breast cancer, pancreatic cancer, polyamines, interfacial polymerization, glioblastomas brain cancer.

INTRODUCTION

While 3-amino-1,2,4-triazole, 3-AT, has been reported to be a possible carcinogen similar ringed compounds that contain the azomethine linkage, -CH=N-, have shown anti-microbial behavior with good pharmacological activity.[1,2]

The importance of inhibiting fungus and bacteria has increased over the past forty years due to the increase in microorganisms that are resistant to common, and often all known drugs. The population that is most at-risk include persons with cancer, recent transplants, and HIV. The main target for antifungal inhibition today is the ergosterol biosynthetic pathway that is important in the production of fungal membranes.[2] A focus for the ergosterol pathway is the cytochrome P-450 dependent 14α-sterol demethylase, CYP51 enzyme, that catalyzes the oxidative removal of the 14α-sterol methyl group of lanosterol giving Δ desaturated intermediates. It has been found that 3-AT derivatives are able to inhibit this pathway and thus there is a major effort at developing new 3-AT related drugs that accomplish this. Further, the clinical value of many of the current antifungal drugs is limited because of their high toxicity and pharmacokinetic problems of these drugs. We have synthesized polymeric organotin drugs that exhibit good inhibition of both bacterial and fungi as well as anticancer behavior.[3-11] The current research is part of this effort.

While much of the current medical focus in on antibacterial and antifungal inhibition we have found that organotin polymeric compounds containing the 3-AT unit also act to inhibit a wide range of human cancer cell lines.[5-11] Since organotin compounds are known to exhibit antibacterial and antifungal activity we hoped to marry these two effects to create compounds that exhibit anticancer activity. This is reported here.

3-Amino-1,2,4-triazole, 3-AT, Figure 1, is a competitive inhibitor of the product of the HIS3 gene, imidazoleglycerol-phosphate dihydratase, IPD.[12,13] IPD catalyzes the sixth step in the production of histidine. 3-AT is also an inhibitor of catalase, an enzyme that converts cellular hydrogen peroxide into water and oxygen.

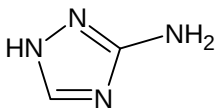

Figure 01: 3-Amino-1,2,4-triazole.

3-AT has been incorporated in a wide variety of metal and non-metal containing polymers for a variety of purposes including as metal organic framework polymers and similar materials.[14-18] A number of polymers have been described containing 3-AT and metal moieties. Lu and coworkers described the formation of 2D metal-organic coordination polymers from the reaction of $Cu(ClO_4)_2$ with 1,2,4-triazole and its derivatives. These metal-

organic frameworks were fluorescence.[14] Silver and cadmium-containing polymers were reported by Pei-Xiu and coworkers from the dehydrogenative coupling reaction involving 3-AT.[15] Non-metal containing polymers have also been reported.[19-21] For example Gloeckner and coworkers described the synthesis of cross-linkable poly[N-(methacryl-2-ethyl)-N'-(3-amino(1,2,4-triazole-2-yl)urea-co-methyl methacrylate] that exhibits complex viscosity changes as heat is added attributed to crosslink formation.[19]

Polymers containing 3-AT have been reported that exhibit biocidal properties. Biocidal polymers were reported formed from reaction of poly(ethylene-alt-maleic acid) with 3-AT.[20] In water the polymers release 3-AT providing the biocidal activity.

Thus, many examples exist where 3-AT is a reactant in the formation of polymers with various potential useful properties including biological and luminance.

To the general public 3-AT is known for its ability to control annual grasses and broadleaf and aquatic weeds. It is sold as aminotriazole, amitrol, and amitrole. It is banned from use by many countries because, as noted before, it is a suspected cancer-causing agent but while it is a suspected cancer-causing agent, as an organotin polyamine polymer it is an anticancer agent able to inhibit a group of human cancer cell lines including breast, pancreatic, and brain cancer. These results are described in this paper along with the characterization of condensation polymers derived from reaction between 3-AT and organotin dichlorides forming polymers as depicted in Figure 2.

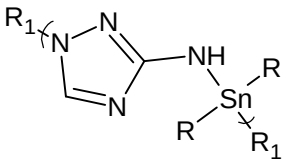

Figure 02: Repeat unit for the product of 3-AT and organotin dihalides where R_1 simply represents chain extension.

EXPERIMENTAL

Synthesis: Reactions were carried out employing the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the 3-AT (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 heptane solution (30 ml) containing the

organotin dihalides (0.00300 mol) was rapidly added (about 3-4 seconds) through a hole in the jar lid using a powder funnel. The resulting solution was blended for 15 seconds. The precipitate was recovered using vacuum filtration and washed several times with deionized water and heptane removing unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

Diphenyltin dichloride (1135-99-5), 3-amino-1,2,4-triazole (61-82-5) 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. They were used as received.

Structural Characterization: Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the products dissolved in dimethyl sulfoxide, DMSO. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a JASCO FT/IR-4100 fitted with an ATR Pro 450-s. ^{1}H 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 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 volts; grid voltage 90% and an acquisition mass range of 500 to 2,500. Fifty to two hundred shots were typically taken for each spectrum. A graphite matrix was employed. Graphite from a number 2 pencil was marked on the sample holder and sample placed onto the graphite mark.

Cell Testing: The toxicity of each test compound was evaluated. Following a 24 h incubation period, the test compounds were added at concentrations ranging to 60 microgram/mL and allowed to incubate at 37°C with 5% CO_2 for 72 h. Following incubation, Cell Titer-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 base-line 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 Eagle's minimal essential medium, 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 one percent, demonstrating that the activity observed was not due to cytotoxicity of any of the diluents used, but was due to activity of the tested compounds. The inhibition curve is sigmoid and the EC_{50} determined at the midpoint of the curve. Once inhibition begins the concentration curve 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 percentage yield and chain length for the organotin dihalide products and 3-AT. The yields and chain lengths are moderate. The products possess good adhesion to glass.

Table 01: Product yield and chain length for the reaction between 3-amino-1,2,4-triazole and organotin dihalides.

Organotin	Yield, %	Molecular Weight	DP
Me_2Sn	48	1.9×10^5	820
Et_2Sn	61	6.7×10^4	260
Bu_2Sn	45	2.2×10^4	70
Oc_2Sn	69	1.6×10^4	45
Ph_2Sn	68	1.7×10^5	1800

There is no trend with respect to percentage yield. The chain length decreases as the alkyl group on the tin increases consistent with the larger alkyl groups interfering with chain growth.

Infrared Spectral Results: Table 2 contains bands and band assignments given in wavenumbers, that is cm^{-1}, for the monomers and polymers from dibutyltin and diphenyltin dichloride There are a number of bands derived from the 3-AT as well as the organotin moiety. For the polymer, there is the large shift in the NH_2 associated bands since these are now NH groups. The ring NH band is now gone since it has lost its proton through bond formation. Bands due to the presence of the ring CH st and C=N are present. There is a new

band about 700 assigned to the formation of the Sn-N linkage. Thus, IR spectroscopy is consistent with the formation of organotin polyamines.

Table 02: Assigned peaks for the monomers and associated polymers derived from reaction with 3-AT and dibutyltin dichloride and diphenyltin dichloride.

Band Assignment	3-AT	Bu_2SnCl_2	Bu_2Sn/ Polymer	Ph_2SnCl_2	Ph_2Sn/ Polymer
NH_2 St	3517		3417		3408
NH St	3499				
NH_2 St	3404		3318		3331
CH Arom St	3326,3261		3307,3227		3331,3214
CH aromatic				3068,3051	3064,3048
CH_3 asym st		2959	2953		
CH_2 asym st		2926	2928		
CH_3 sym st		2872	2870		
CH_2 sym st		2858	2854		
NH Bend	1622				
C=N Ring St	1536		1538		1537
Sn-Ph st				1480	1481
C=C st				1432	1428
Sn-Ph st				1071	1061
C-C st		1178,1152	1192,1150		1188,1151
Ring breathing				996	997
CH Torsion	824		831		815
Sn-N			700		719

3-AT band assignments are based on Pagacz-Kostrzewa and coworkers.[22] Organotin bands assignments are based on the work of Carraher and coworkers.[23-32]

MALDI MS Analysis

Regular MALDI MS requires that the matrix and polymer are soluble in a volatile liquid that allows intimate mixing of the matrix that absorbs the radiation necessary to make it air born taking with it the sample compound. It is like wrapping the sample compound as a pupa of a butterfly in the chrysalis with the matrix supplying the necessary energy to take the entombed sample air born as well as protecting the sample from premature degradation. This requirement excludes most typical polymers from successful entire chain MALDI MS analysis because of the lack of polymer solubility in such volatile liquids. We have been investigating the solid-state fragmentation of various polymers employing MALDI MS emphasizing metal-containing polymers for use in the structural identification of these polymers. In this approach the emphasis is on investigating the fragments created by polymer chain scission.

In the present study MALDI MS was carried out on the products over the general range of 500 to 8000 Da. All mass values are given in Da. The technique as employed by us has been recently reviewed.[28][33-35] Here is described the MALDI MS results for two organotin/3-AT polymers.

Recently we have been employing graphite as the matrix material because it gives good results with few interfering ion fragments produced above 500 Da mass which is the typical lower mass range employed in our studies.[28] Two general MALDI MS modes were employed. These were the reflective and linear modes. The reflective mode has a longer focal length than the linear mode. Results for the reflective mode allow finer features, such as isotopic abundances, to be more accurately determined but generally results in the detection of lower masses. By comparison, the linear mode has a shorter flight distance and results in the detection of higher masses. Sodium is a usual contaminant.

Figure 3 contains a portion of the MALDI MS for the dibutyltin/3-AT polymer for the refractive mode. Figure 4 contains a similar MALDI MS but using the linear mode.

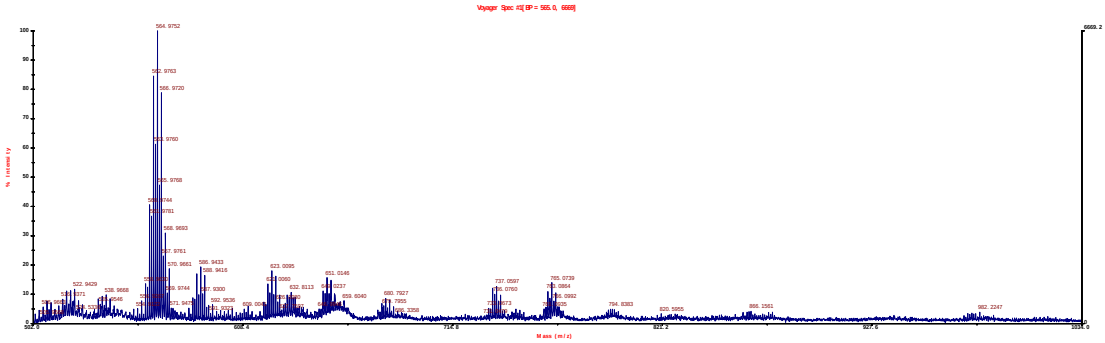

Figure 03: MALDI MS for the polymer from dibutyltin dichloride and 3-AT using the refractive mode over the approximate range of 600 to 1000 Da.

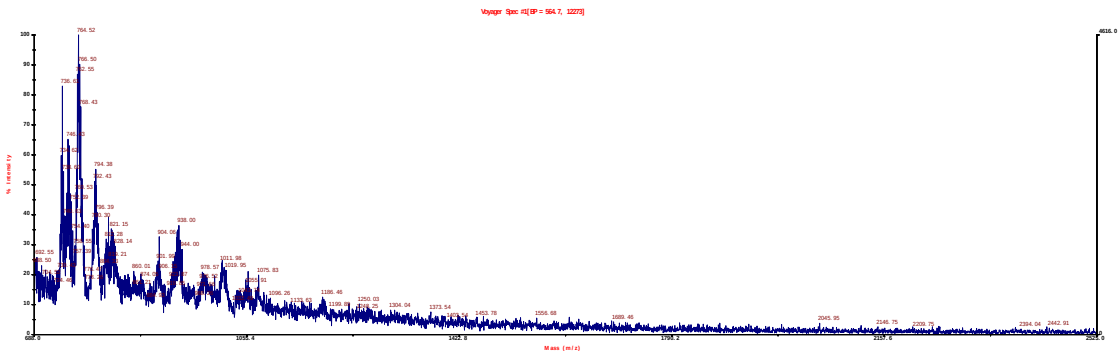

Figure 04: MALDI MS for the polymer from dibutyltin dichloride and 3-AT using the linear most over the approximate range of 600 to 2500 Da.

Table 3 contains results for the polyamine derived from dibutyltin dichloride and 3-AT for both the linear and reflective modes using graphite as the matrix. The abbreviation AT is employed for 3-AT minus two protons; Bu is butyl.

Table 03: Most abundant ion fragment clusters derived from the product of dibutyltin dichloride and 3-AT using graphite as the matrix.

Ion Frag Cluster; Linear, Da	Ion Frag Cluster, Reflective, Da	Tentative Assignment
	523	U+BuSn,Na
	538	U+BuSn,Na,NH
	565	U+Bu_2Sn,NH
	587	2U-Bu+NH
	623	2U
645	651	2U,NH
683		2U+AT-2NH
707	701	2U+AT
	737	2U+AT,Na
	765	2U+Sn,NH
	794	2U+Sn,NH,Na
808		2U+BuSn
846		2U+BuSn,Na,NH
	866	2U+NH,Na
	982	3U+NH,Na
1009		3U+AT-Bu
1072		3U+AT
1142		3U+BuSn,Na
1180		3U+ Bu_2Sn
1221		4U-Bu+NH
1256		4U
1294		4U,Na
1347		4U+AT
1371		4U+AT,Na
1401		4U+Sn,NH
1465		4U+BuSn,Na
1557		4U+BuSn, Na
1689		5U+AT,Na
2147		6U+BuSn,AT
2210		7U
2394		7U+BuSn
2443		7U+Bu_{2Sn}

As expected the linear mode gives ion fragments to a higher mass range than does the reflective mode. The reflective mode gives ions to three repeat units while the linear mode gives ion fragments to seven repeat units. Both ranges give ion fragment clusters consistent with the repeat unit shown in Figure 2. The ion fragments typically show no fragmentation of the 3-AT unit other than release of the amine moiety. This is consistent with the mild nature of MALDI MS. The loss of the butyl group from the dibutyltin moiety occurs and is common for dibutyltin-containing polymers.

Tin contains isotopes of which seven have a relative isotopic abundance of five percent and greater. The presence of tin within the ion clusters is indicated by the "tell-tale" fingerprint caused by the isotopic abundance of these tin isotopes. Table 4 contains isotopic matches for ion fragment clusters centering about 565 and 587 Da. The agreement with the standard is reasonable and consistent with these ion fragment cluster containing two tin atoms.

Table 04: Isotopic abundance match for two ion fragment clusters containing two tin atoms derived from the product of dibutyltin dichloride and 3-AT using graphite as the matrix. (Only ion fragments >5% relative abundance are reported.)

Standard		U+Ph_2Sn,Na		2U+Na	
Da	**% Rel Abu**	**Da**	**% Rel Abu**	**Da**	**% Rel Abu**
232	12	559	14	581	10
233	13	560	15	582	12
234	43	561	45	583	45
235	35	562	37	584	36
236	94	563	90	585	92
237	51	564	52	686	53
238	100	565	100	587	100
239	35	566	34	588	35
240	81	567	79	589	83
242	32	569	32	591	34
244	22	571	20	593	22

In each case, ion fragment clusters given in Table 3 are consistent with the presence of tin with tin being the site for bond scission. Presumably, the ion fragment clusters are produced through the bond scission at tin atom sites resulting in the formation of these lower mass ion fragment clusters as shown in Figure 5.

Table 5 contains the most abundant ion fragment clusters for the product of 3-AT and diphenyltin dichloride. Chain lengths to two units are found for the reflective mode and to 18 units for the linear mode.

Table 05: Most abundant ion fragment clusters derived from the product of diphenyltin dichloride and 3-AT using graphite as the matrix.

Ion Frag Cluster;Linear, Da	Ion Frag Cluster, Reflective, Da	Tentative Assignment
635	631	U+Ph_2Sn
647	651	U+ Ph_2Sn,Na
672	672	U+Ph_2Sn,NH,Na
712	712	2U
738	739	2U+Na
750	749	2U,NH,Na
	836	2U+Sn
845	845	2U+Sn,NH
890	894	2U+Sn,2NH,Na
926	931	2U+ PhSn,NH
997		2U+ Ph_2Sn,NH
1076		3U
1175		3U+AT,Na
1270		3U+PhSn
1476		4U+NH,Na
1712		5U-Ph
1992		5U+ Ph_2Sn,NH
2159		6U+Na
2399		6U+ Ph_2Sn
2600		6U+AT,Na
2766		7U+ Ph_2Sn
2995		8U+Sn,Na
3194		9U-NH
3350		9U+Sn,Na
3539		10U-NH
3936		11U+Na
4343		12U+AT-NH
4722		13U+AT
5127		14U+Sn,Na
5471		15U+Sn
6451		18U+NH,Na

Table 6 contains isotopic abundance matches for two ion fragment clusters containing two tin atoms. The agreements are reasonable and consistent with the presence of two tin atoms in these ion fragment clusters.

Table 06: Isotopic abundance match for two ion fragment clusters containing two tin atoms derived from the product of diphenyltin dichloride and 3-AT using graphite as the matrix. (Only ion fragments >5% relative abundance are reported.)

Standard		U+Ph_2Sn,Na		2U+Na	
Da	% Rel Abu	Da	% Rel Abu	Da	% Rel Abu
232	12	647	10	733	11
233	13	648	12	734	12
234	43	649	40	735	41
235	35	650	35	736	35
236	94	651	88	737	90
237	51	652	51	738	51
238	100	653	100	739	100
239	35	654	33	740	35
240	81	655	81	741	83
242	32	657	30	743	30
244	22	659	21	745	22

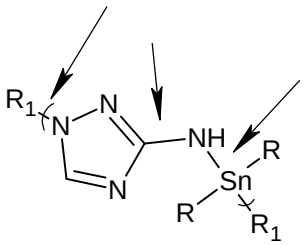

Figure 5: Repeat unit illustrating the major sites for bond scission.

In summary MALDI MS was conducted on the polyamine products of organotin dihalides and 3-AT employing graphite as the matrix material. Ion fragments to two and three units containing 3 and 4 tin atoms are found employing the reflective mode for the dibutyltin and diphenyltin polymers but to seven tin unit-containing ion fragment clusters for the dibutyltin polymer and 18 units for the diphenyltin polymer using the linear mode. The isotopic abundance pattern is consistent with the presence of tin atoms in the ion fragment clusters. These results are also consistent with the proposed polymer structure repeat unit.

Proton NMR Spectroscopy: Proton NMR spectroscopy was carried out on the products and monomers. 3-AT shows bands at 12.2 for the internal NH, 7.6 for the amine NH_2 and 5.8 for the ring hydrogen.[36-38] All polymers show a band 7.4 assigned to the amine NH group, and a band about 5.8 from the ring proton. The ring NH is absent consistent with the linkage with the organotin moiety. Thus, the NMR results are consistent with the proposed structure. NMR assignments for the organotin dihalides and associated polymers are taken from the literature.[29-32] Dimethyltin dichloride shows a band at 1.2. The dimethyltin polymer shows a

band at 1.2. diethyltin dichloride shows two bands, one at 1.1 and one at 1.25. The polymer shows two bands, one at 1.25 and one at 1.22. Thus, NMR spectroscopy is consistent with the presence of both reactant moieties and absence of the internal NH as expected. Because of the poor polymer solubility in d6 DMSO further analysis is not viable.

Tumor Analysis: Much of our recent activity has focused on the synthesis and preliminary cancer cell line testing of various organotin-containing polymers for the purpose of investigating the various abilities to inhibit the growth of human cancer cell lines as a function of the particular polymer structure.[23-27][29-32] The recent emphasis has been on pancreatic cancer[39] and gioblastoma brain cancer[40] because there exists no cure for either once they spread. Table 7 contains the cell lines employed in the current study.

Table 07: Cell lines employed in the current study.

Strain Number	NCI Designation	Species	Tumor Origin	Histological Type
3465	PC-3	Human	Prostate	Carcinoma
7233	MDA MB-231	Human	Pleural effusion breast	Adenocarcinoma
1507	HT-29	Human	Recto-sigmoid colon	Adenocarcinoma
7259	MCF-7	Human	Pleural effusion-breast	Adenocarcinoma
ATCC CCL-75	WI-38	Human	Normal embryonic lung	Fibroblast
CRL-1658	NIH/3T3	Mouse	Embryo-continuous cell line of highly contact-inhibited cells	Fibroblast
	U251	Human	Glioblastoma multiforme	Astrocytomas
	G55	Human	Glioblastoma	Astrocytomas
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Two cell lines are typically used as “standard” cell lines employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These cell lines are the NIH/3T3 and WI-38 cell lines. NIH/3T3 cells are mouse embryo fibroblast cells. They are cell lines that are referred to as partially transformed cells since unlike normal cells they are immortal, but they retain other characteristics of normal cells such as being contact-inhibited. Relative to most normal cells they are robust and easily maintained.

WI-38 cells are normal embryonic human lung fibroblast cells with a finite life time of about 50 replications. Compared to NIH/3T3 cells they are more fragile and difficult to maintain for long periods of time. Thus, NIH/3T3 cells are often favored because of ease of handling aided by an infinite life span.

Different measures have been employed in the evaluation of cell line results. The most widely used involves the concentration dose needed to reduce the growth of the particular cell line. The term effective concentration, EC, is employed here. 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 8 contains the EC_{50} values found for the monomers and polymers and include values for cisplatin as a standard. Cisplatin is a widely used anticancer drug included in the treatment of neck and head cancer, cervical cancer, breast cancer, bladder cancer, lung cancer, mesothelioma, brain tumors, ovarian cancer, testicular cancer, and neuroblastoma.[41] It is quite toxic with many unwanted side effects.[41]

In past studies, as the organotin dihalide is varied for a particular Lewis base, the polymer containing the dibutyltin moiety showed the greatest ability to curtail cancer cell line growth followed by those containing the diphenyltin moiety. In the current study there appears no major trend with respect to the ability to inhibit the cancer cell lines. Even so, the dibutyltin moiety does show good inhibition towards all of the cell lines. In all cases the general variation of the toxicity range is relatively small as the organotin moiety is varied. Thus, there exists no clear general trend with respect to the nature of the organotin moiety with all of the polymers showing decent inhibition of all of the human cancer cell lines.

As noted, the dibutyltin polymers do exhibit decent inhibition of all the cancer cell lines. This is important since dibutyltin dichloride is the least expensive of the organotin halides and available in ton and greater amounts.[42] Because of its widespread commercial use, more is known about it than any of the other organotin monomers. It is the least toxic to humans of the organotin monomers.[41] Finally, in nature it degrades to simple tin oxide offering a low toxic form of degradation product.[41] Even so, while it is widely employed as a paint additive, if it is used in marine coatings it is required to be in polymeric form since it leaches and into waterways and is found to limit sea life growth.

As noted, our current focus in on pancreatic and brain cancer because there is no cure once it metastasizes prior to detection. In the USA about 32,000 individuals are yearly found with pancreatic cancer. Most die within less than a year. Worldwide, it is the fourth leading cause of cancer death. We have found a number of organotin polymers that exhibit good ability to inhibit pancreatic cancer. In the current study we use the two most widely studied human pancreatic cancer cell lines. The tested cell lines are AsPC-1 which is an adenocarcinoma

pancreatic cell line, which accounts for about 80% of the diagnosed human pancreatic cancers, and PANC-1 which is an epithelioid carcinoma pancreatic cell line, accounting for about 10% of the human pancreatic cancer cases. For the current study, the organotin polymers show decent inhibition of both cell lines. This inhibition is similar in both of the two cell lines consistent with the idea that the polymers may offer broad-spectra inhibition of other pancreatic cancer cell lines.

Our second concentration is on the inhibition of brain cancers. As with pancreatic cancer, the typical prognosis is not favorable with a five-year survival rate in the USA of about one third. In the USA there are about 44,000 new cases of brain tumors (2005) accounting for about 2.5% of the cancer-related deaths. Glioblastomas tumors typically have poor outcomes while meningiomas typically have favorable end points. It is glioblastoma cancer that is our initial focus. There are a variety of brain cancer tumors that have been employed. The U251 is among the most often used. It was established at the Wallenberg laboratory, Uppsala, Sweden about 40 years ago derived from human gliomas, derived from a male patient with malignant astrocytoma. G55 is a human glioblastoma (very aggressive) cell line that has been passed through nude mice and re-established as a stable xenograft cell line. The two cell lines each have unique levels of ATP and respond differently to assays with different tumorgenicity, mutations, and expressions of different genes. Studies show that G55 tends to be more invasive some believing that G55 models are more physiologically relevant because of greater invasiveness and migration since they form invasive intracranial tumors in rodents more characteristic of primary human GBM. There is no decent chemo treatment for brain cancer thus the ability to inhibit it by treatment with chemo drugs is greatly needed. The current organotin polymers show ability to inhibit both types of brain cancer cell lines to a similar extent (Table 8).

Table 08: EC_{50} Concentrations (micrograms/mL) for the tested compounds derived from various organotin polymers, monomers, 3-AT and cisplatin. Values given in ( ) are Standard Deviations for each set of measurements.

Sample	3T3	*WI-38*	*PANC-1*	*AsPC-1*	*PC-3*
Me_2SnCl_2	0.43 (.1)	0.22(.1)	0.80(.1)	0.71(.1)	0.51(.1)
Me_2Sn/AT	0.91(.4)	0.75(.4)	1.4(.4)	1.4(.4)	1.4(.4)
Et_2SnCl_2	0.46(.1)	0.20(.1)	0.48(.1)	0.90(.1)	0.61(.1)
Et_2Sn/AT	1.1(.3)	0.81(.2)	1.2(.2)	1.3(.2)	1.5(.5)
Bu_2SnCl_2	0.20 (.05)	0.20(.05)	0.0032(.001)	0.012(.01)	1.4(1.1)
Bu_2Sn/AT	0.95(.3)	0.80(.2)	1.4(.2)	1.3(.2)	1.3(.5)

Oc_2SnCl_2	0.56(.1)	0.30(.1)	0.85(.1)	0.85(.1)	0.55(.1)
Oc_2Sn/AT	0.98(.2)	0.71(.3)	1.2(.3)	1.4(.4)	1.4(.3)
Ph_2SnCl_2	0.66(.1)	0.25(.1)	0.71(.1)	0.83(.1)	0.82(.1)
Ph_2Sn/AT	0.96(.3)	0.76(.2)	1.5(.2)	1.3(.4)	1.3(.2)
3-AT	>2.0	>2.0	>2.0	>2.0	>2.0
Cisplatin	0.015(.01)	0.012(.01)	0.0023(.005)	0.0035(.005)	0.0044(.004)

Sample	***MDA-MB-231***	***HT-29***	***MCF-7***	***U251***	***G55***
Me_2SnCl_2	0.44(.1)	0.56(.1)	0.66(.1)	0.91(.5)	1.2(.6)
Me_2Sn/AT	1.3(.5)	1.4(.4)	1.5(.4)	1.4(.3)	1.3(.5)
Et_2SnCl_2	0.64(.1)	0.71(.1)	0.77(.1)	1.1(.6)	1.3(.6)
Et_2Sn/AT	1.4(.5)	1.2(.5)	1.2(.5)	1.4(.5)	1.3(.4)
Bu_2SnCl_2	1.4(1.3)	1.2(.1)	0.70(.06)	1.0(.2)	1.0(.4)
Bu_2Sn/AT	1.3(.5)	1.3(.3)	1.4(.2)	1.3(.2)	1.4(.4)
Oc_2SnCl_2	0.65(.1)	0.65(.1)	0.70(.1)	1.3(.7)	0.95(.6)
Oc_2Sn/AT	1.2(.4)	1.4(.5)	1.4(.5)	1.2(.3)	1.2(.5)
Ph_2SnCl_2	0.76(.1)	0.56(.1)	0.68(.1)	0.89(.6)	0.97(.6)
Ph_2Sn/AT	1.3(.5)	1.2(.2)	1.3(.2)	1.4(.5)	1.3(.4)
3-AT	>2.0	>2.0	>2.0	>2.0	>2.0
Cisplatin	0.0029(.002)	0.0041(.003)	0.0057(.003)	0.015(.01)	0.020(.01)

While not a primary focus, breast cancer is a cancer of great concern.[23] 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 negative while the MCF-7 (strain line 7259) cells are estrogen receptor (ER) positive. In some studies, involving organotin and other metal-containing polymers, there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure.[23] Specifically, in studies where the polymer contains a Lewis base possessing the O-Phenylene moiety, such as hydroquinone and hydroquinone derivatives and diethylstilbestrol that is employed in certain breast hormone therapies, there was a greater ability to inhibit the MDA-MB-231 cells in comparison to the MCF-7 cells presumably because the MCF-7 cells react with the drugs removing them from inhibiting the MCF-7 cells whereas those structures, such as the 1-AT in the present study, that do not contain this structural moiety showed little difference between the ability to inhibit the two cell lines. The PC-3 (3465) cells are also of interest because this particular prostate cell line is viewed as among the most resistant of the prostate cancer cell lines. They are also effectively inhibited by the organotin polymers.

Another measure of the potential use of compounds as an effective anticancer agent is the concentration of drug necessary to inhibit standard cells compared to the concentration of drug necessary to inhibit the growth of the test cell line. Again, a variety of symbols are

employed to describe similar calculations. Here, the term chemotherapeutic index, CI, is used. Values from WI-38 are considered to more closely match live animal studies so it is employed in Table 9. Thus, the CI_{50} is the ratio of the EC_{50} for the WI-38 cells divided by the EC_{50} for the particular test cell. Values greater than one are desirable in this measure since it indicates that there is a preference for inhibiting the cancer cell lines in comparison to the standard cells. For the brain cell lines, the CI_{50} values for the monomers and polymers are generally greater than the standard cisplatin. Thus, while cisplatin is employed to treat neuroblastoma the organotin/AT polymers show a similar ability to inhibit the brain cancer cell lines based on their CI_{50} but not their EC_{50} values. Also, the 3-AT polymers are much less toxic based on the WI-38 values compared to cisplatin.

Table 09: CI_{50} values for monomers and polymers derived from data given in Table 8 based on WI-38 data.

Sample	EC_{50}WI-38/ EC_{50}PNC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}MDA
Me_2SnCl_2	0.28	0.31	0.43	0.50
Me_2Sn/AT	0.54	0.54	0.54	0.54
Et_2SnCl_2	0.83	0.81	0.91	0.91
Et_2Sn/AT	0.68	0.61	0.54	0.58
Bu_2SnCl_2	63	18	0.14	0.14
Bu_2Sn/AT	0.57	0.62	0.62	0.57
Oc_2SnCl_2	0.35	0.35	0.55	0.46
Oc_2Sn/AT	0.59	0.51	0.51	0.55
Ph_2SnCl_2	0.35	0.31	0.30	0.33
Ph_2Sn/AT	0.51	0.58	0.58	0.58
Cisplatin	5.2	3.4	2.7	4.1

Sample	EC_{50}WI-38/ EC_{50}MCF-7	EC_{50}WI-38/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}U251	EC_{50}WI-38/ EC_{50}G55
Me_2SnCl_2	0.39	0.39	0.45	0.31
Me_2Sn/AT	0.54	0.50	0.54	0.58
Et_2SnCl_2	0.71	0.67	0.34	0.29
Et_2Sn/AT	0.68	0.68	0.59	0.62
Bu_2SnCl_2	0.29	0.17	0.33	0.33
Bu_2Sn/AT	0.62	0.58	0.62	0.58
Oc_2SnCl_2	0.43	0.46	0.34	0.46
Oc_2Sn/AT	0.51	0.51	0.59	0.59
Ph_2SnCl_2	0.37	0.45	0.29	0.30
Ph_2Sn/AT	0.63	0.58	0.54	0.58
Cisplatin	2.1	2.9	0.80	0.57

CONCLUSIONS

Polyamines from reaction of 3-AT and organotin dichlorides were produced in moderate yield and chain length employing the interfacial polymerization system using commercially available reactants. Chain length decreased as the steric nature of the organotin alkyl group increased consistent with the increased size interfering with the polymer forming step. IR spectral analysis showed the loss of the ring-NH group as expected because of the formation of the Sn-AZ linkage. Formation of a new band assigned to formation of the Sn-AT linkage is found. NMR was also consistent with polyamine formation with the loss of the NH ring proton. MALDI MS shows ion fragment clusters to 18 units for the diphenyltin polymer and good isotopic abundance matches for the ion fragment clusters. All of the polymers show good inhibition of a group of human cell lines including breast, lung, prostrate, pancreatic and glioblastoma human cancer cell lines signaling these polymers to be a promising new group of anticancer agents.

REFERENCES

1. Issa Y, Hassib H, Abdelaal H. ^{1}H NMR, ^{13}C NMR and mass spectral studies of some Schiff bases derived form 3-amino-1,2,4-triazole. Molecular and Biomolecular Spectroscopy, 2009; 74: 902-910.
2. Ozkirimli S, Kazan F, Tunalin Y. Synthesis, antibacterial and antifungal activities of 3-(1,2,4-triazol-3-yl)-4-thiazolidinones. J. Enzyme Inhibition Med. Chem, 2009; 24: 447-452.
3. Carraher CE. Novel compounds for control of microorganism. US Pat. 5,043,463; 1990.
4. Carraher CE, McNeely C, Schroeder J, Giron D. Cellulose products having enhanced thermal, solvent and biological properties. U.S. Pat. 4,312,981; 1982.
5. Carraher CE, Butler C. Materials and methods for controlling methicillin-resistant Staphylococcus aureus microbes. US Pat. 5,840,760; 1996.
6. Carraher CE, Giron D. Antimicrobial properties of metal-containing polymers. Progress in Chemical Disinfection, 1984; 63-76.
7. Carraher CE, Butler C, Reckleben L. Treatment of Yeast Infections Employing Organotin Containing Modified Poly(vinyl Alcohol). Cosmetic and Pharmaceutical Applications of Polymers, 1991; 255-261.
8. Carraher CE, Butler C, Naoshima Y, Sterling D, Saurino V. Factors Affecting the Bacterial Activity of Saccharides and Polysaccharides Modified through Reaction with Organostannanes. Biotechnology and Bioactive Polymers, 1994; 2-8.

9. Carraher CE, Sterling D, Butler C, Ridgway T. Organostannane-Modified Kraft Lignin in the Selected Treatment of Yeast-Causing Microorganisms. Biotechnological Polymers, 1994; Chapter 13.
10. Carraher CE, Naoshima Y, Nagao K, Mori Y, Barot G, Battin A. Bacterial Inhibition by Organotin Polymers, Inorganic and Organometallic Macromolecules: Design and Application. NY: Springer, 2008.
11. Carraher CE, Naoshima Y, Nagao K, Roner MR, Zhao A, Barot G, Battin A. Antibacterial, Yeast and Fungal Polymeric Material Derived from Organotin-Containing Materials. J. Polym. Mater, 2011; 28: 303-336.
12. Merck Index, 15th Edition, Merck & Company, NJ: Rahway, 2013.
13. Joung JK, Ramm EI, Pabo CO. Bacterial two-hybrid selection system for studying protein-DNA and protein-protein interactions. Proc. Natl. Acad. Sci, 2000; 97: 7382-7387.
14. Xu L, Zhang XCh, Liu WG, Liu B. 2DBitriazole-bridging copper(II) polymer: Hydro thermal synthesis, crystal structure, and fluorescence. J. Structural Chem, 2012; 53(1): 179-183.
15. Pei-Xiu Y, Jian-Kai C, Zhao-Ji L, Ye-Yan Q, Jian Z, Yuan-Gen Y. Ag(I)-mediated in situ dehydrogenative coupling of 3-amino-1,2,4-triazole into 3,3'-azobis(1,2,4-triazole) in Ca(II) coordination polymers. Inorganic Chemistry, 2009; 48(23): 10859-10861.
16. Jie-Peng Z, Yan-Yong L, Wei-Xiong Z, Xio-Ming C. Temperature- or guest-induced drastic single-crystal-to-crystal transformations of a nonporous coordination polymer. J. Amer. Chem. Soc, 2005; 127(41): 14162-14163.
17. Gao JY, Wang N, Xiong X, Xiong XH, Chem CJ, Xie WP, Ran XC, Cai YP. Structures, luminescence and magnetic properties of three 3D lanthanide polymers based on 3-amino-1,2,4-triazole and oxalate. Inorganic Chem. Communications, 2013; 37: 197-201.
18. Luan F, Xu L, Gao Y, Liu B. 3D nitrate-bridging heterometal-azido inorganic polymer containing Cd8 cube: synthesis, structure, topology and fluoresce. Korean Chemical Soc, 2011; 32(5): 1769-1772.
19. Gloeckner P, Osterhold M, Ritter H. Synthesis of cross-linkable poly[N-(methacryl-2-ethyl)-N'-(3-amino(1,2,4-triazole-2-yl)urea-co-methyl methacrylate]. Macromolecules, 2002; 35(6): 2050-2054.
20. Hartmann M, Bauer H, Wermnann K. Biocide polymer: modification of poly(ethylene-alt-maleic acid) with 3-amino-1,2,4-triazole and 3(5)-methylpyrazole. Zeitschrif Fuer Chemie, 1986; 26(10): 369-371.

21. Sinirlioglu D, Asian A, Muftuoglu A, Ekrem A, Bozhurt A. Synthesis and proton conductivity studies of methacrylate/methacrylate polymer electrodes. J. Applied Polym. Sci, 2013; 131: 39915/1-39915/8.
22. Pagacz-Kostrzewa M, Bronisz R, Wierzejewska M. Theoretical and matrix isolation FTIR of 3-amino-1,2,4-triazole. Chem. Physics Letters, 2009; 473: 238-246.
23. Carraher CE, Roner MR. Organotin polymers as anticancer and antiviral agents. J. Organomet. Chem, 2014; 751: 67- 82.
24. Zhao A, Shahi K, Roner MR, Barot G, Fiore T, Pellerito C, Scopelliti M, Pellerito L, Carraher CE. Ciprofloxacin polymers derived from diallyltin and divinyltin dihalides. J. Polym. Sci, 2008; 25: 87-109.
25. Carraher CE, Gupta A, Roner MR. Organotin polyamine esters from reaction of 6-aminopenicillanic acid and organotin dihalides. J. Polym. Mater, 2012; 29: 377-390.
26. Carraher C, Lanz L. Synthesis and initial structural characterization of organotin polymers containing norfloxacin. J. Polym. Mater, 2003; 20: 91-100.
27. Carraher CE, Li F, Butler C. Synthesis and structural characterization of polymers derived from the condensation of organotin dichlorides and the synthetic amino acids p-aminobenzoic acid and ampicillin. J. Polym. Mater, 200; 17: 377-384.
28. Carraher CE, Suresh V, Roner MR. Self-matrix activity of organotin polyether ester polymers containing alpha-cyano-4-hydroxycinnamic acid. JCAMS, 2015; 3: 32-44.
29. Carraher CE, Roner MR, Campbell A, Moric-Johnson A, Miller L, Slawek P, Mosca F, Einkauf J, Haky J, Crichton R. Synthesis of organotin polyesters from reaction of the salt of D-camphoric acid and organotin dihalides and initial anticancer activity. J. Inorg. Organomet. Polym. Mat, 2018; 28: 481-491.
30. Carraher CE, Roner MR, Patel D, Miller L, Moric-Johnson A, Slawek P, Mosca F, Frank J. Synthesis of organotin polymers from 2-ketoglutaric acid and their ability to inhibit the growth of human cancer cell lines. Hematology Medical Oncology, 2018; 3(1): 1-9.
31. Carraher CE, Roner MR, Sookdeo N, Moric-Johnson A, Miller L, Johnson J. Synthesis and initial cancer cell results of organotin polyethers derived from the anticoagulant dicumarol. Int. J. Applied Pharm. Biological Res, 2017; 2(5): 1-17.
32. Carraher CE, Slawek P, Roner MR, Moric-Johnson A, Miller L, Einkauf J, Russell F. Synthesis and structural and initial cancer cell line characterization of organotin polyesters from dipicolinic acid. J. Inorg. Organometal. Polym. Chem, 2016; 26: 1338-1350.

33. Carraher CE, Suresh V, Roner MR. Graphite as a Matrix for Organotin Polymers. J. Polym. Mater, 2015; 32: 151-168.
34. Carraher CE, Roner MR, Carraher CL, Crichton R, Black K. Use of mass spectrometry in the characterization of polymers emphasizing metal-containing condensation polymers. J. Macromol. Sci. A, 2015; 52: 867-886.
35. Carraher CE, Sabir T, Carraher CL. Fundamentals of Fragmentation Matrix Assisted Laser Desorption/Ionization Mass Spectrometry, NY: Springer, 2008.
36. Issa Y, Hassib H, Abdelaal H. ^{1}H NMR, ^{13}C NMR and mass spectral studies of some Schiff bases derived from 3-amino-1,2,4-triazole. Spectrochimica Acta Part A: Mol. Biomolecular Spect, 2009; 74: 902-910.
37. Freiberg W, Kroger C. NMR-Spectra 3-substituierter 1,2,4,-triazol. Tetrahedron Letters, 1967; 22: 2109-2112.
38. Barlin G, Batterham T. Proton magnetic resonance spectra of some diazoles, triazoles, and tetrazoles. J. Chem. Soc. B, 1967; 516-518.
39. Carrher CE, Roner MR, Frank J, Slawek P, Mosca F, Shahi K, Moric-Johnson A, Miller L. Organotin polymers for the control of pancreatic cancer. OBM Hepatology & Gastroenterology, 2019; 3(2): 1-10.
40. Carraher CE, Roner MR, Frank J, Mosca F, Slawek P, Miller L. Inhibition of human glioblastomas brain cancer cell lines by metal-containing polymers. World Journal of Pharmaceutical Research, 2019; 8(6): 123-139.
41. Oun R, Moussa YE, Wheate NJ. Side-effects of platinum-based chemotherapy. Dalton Trans, 2018; 47: 6645-6653.
42. Hoch M. Organotin compounds in the environment-an overview. Appl. Geochem, 2001; 16: 719-743.