

Group 15 Organotin Containing Polyamines from Histamine and Their Ability to Inhibit Cancer Cell Lines from Pancreatic, Breast and other Cancers

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Abstract

The Polyamines of Group 15 metals are synthesized giving low yield and moderate to high molecular weight employing commercially available reactants and the interfacial polycondensation system. IR spectroscopy shows formation of M-N bands. MALDI MS shows the ion fragments to three units long with isotopic abundance results showing antimony in the triphenylantimony-derived clusters.

The absence of the histamine ring proton in Nuclear magnetic resonance is consistent with formation of the polyamine structure. In tumor analysis polymers inhibit growth of cancer cell line with the triphenylarsenic/histamine polymer exhibiting outstanding ability for the inhibition of PANC-1 pancreatic cell line with the best Cl_{50} values thus far found.

Keywords: Group 15 polymers; Pancreatic cancer; Histamine; Interfacial polymerization; MALDI MS; Breast cancer

Introduction

We have been synthesizing metal-containing polymers for a number of reasons. Most recently our focus has been on synthesis of polymers to combat unwanted pathogens and infectious agents. Here the focus is cancer. Our rationale involves the synthesis of metal containing moieties with known biological activities with Lewis bases with biological activities hoping for a synergetic affect. The Group 15 elements have known biological activities, for instance [1-4]. The topic of group 15 elements containing polymers has recently been reviewed which are synthesized from simple reactions of organometallic dihalides or dinitrates with Lewis bases, ex: Diamines and Diols [4-14]. Hereafter stated to emphasize Group-15 containing polymers exhibiting inhibitions of pancreatic, breast, prostate, lung and other cancer cell lines [15-19].

Histamine (2-aminoethylimidazole; **Figure 1**) is involved in complex biological processes where it interacts with specific receptors on cell membranes [20-24]. Histamine is believed involved with a variety of human pathologies including asthma, allergies, acid-peptic disorders, and duodenal ulcers. It is an amino acid that is a neurotransmitter or neuromodulator largely synthesized in mast and white blood cells. It increases the permeability of capillaries in white blood cells increasing their ability to act as a defense to certain pathogens. Hence it fits our criteria of exhibiting some biological activity on its own.

A number of reports describe incorporating the histamine moiety into polymers. Most of these involved two approaches. The first approach is the coordination of histamine or histamine-containing molecules with various metal ions. For instance Modder, Koten and others reported on the formation of a number of coordination polymers formed by binding of peptide-based polydentate ligands through a self-assembly process containing the histamine moiety using silver and copper ions [25]. There were a variety of structures formed depending on reaction conditions. Jeong, Ha and coworkers described the formation of Langmuir-Blodgett films from the coordination of potassium, iron, and other ions onto the polymer poly(N-(2-(4-imidazolyl)ethyl)maleimide-alt-1-octadecene) that has the histamine moiety "hanging" from the main polymer chain [26]. The films have decent stability withstanding a surface pressure of 40 to 50 mN/m. Self-assembly ladder-like coordination polymers formed through reaction with copper+2 were reported by Min and Suh [27]. The products show ferromagnetic interactions dependent on the particular part of the chain measured.

The second approach concerned incorporating the histamine moiety as a side-unit on an already existing polymer. Much of this work was aimed at developing molecules of potential biological importance. Anderson, Lynn, and Langer formed a number of cationic polymers for gene delivery including those containing the histamine moiety using a semi-automated synthetic approach [28]. Combinatorial, automated high-throughput synthesis was employed to synthesize and screen the products. Nelson, Lavigne and coworkers synthesized a

number of polymers from the reaction of diamine-containing reactants, including histamine, with reaction through the acid group on carboxylic acid-substituted poly(thiophene) [29]. Wong and Putnam also incorporated the histamine moiety on the side-chain of a polymer, in this case polymethacryloxysuccinimide [30]. The effort studied the delivery of histamine and other side-chain units. Chen and coworkers studied the effect of proton transport by supramolecular nanochannels in comb polymers including histamine units [31].

This paper describes the synthesis of Group 15-containing polyamines from reaction between the histamine and triphenylmetal dihalides (**Figure 2**).

Experimental

Synthesis

The interfacial polycondensation technique was employed to synthesize the polymers. Initially, histamine (0.00200 mole) and sodium hydroxide (0.0040 mole) dissolved in water (20 mL) were added to a one quart Kimax emulsifying jar. The jar was 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 (about 18,000 rpm) was begun and a chloroform solution (20 ml) for the triphenyl antimony and triphenyl arsenic and carbon tetrachloride for the triphenyl bismuth solution added (about 3-4 seconds) through a hole in the jar lid using a powder funnel. Blending continued for 30 seconds. Vacuum filtration was used to recover the precipitate which was then washed several times with deionized water and chloroform or carbon tetrachloride to remove unreacted materials and unwanted by-products. The solid was transferred to a glass Petri dish and allowed to dry.

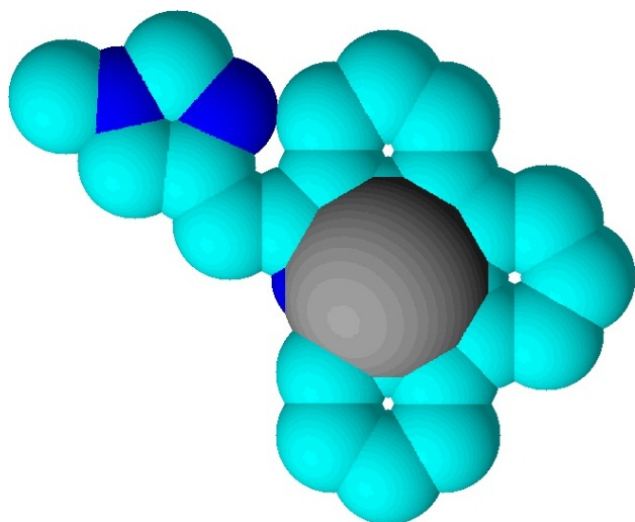


Figure 1. Space filled model of triphenylantimony dichloride and histamine.

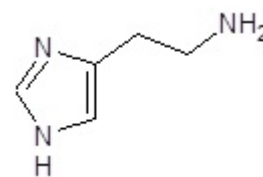


Figure 2. Chemical Structure of histamine.

Triphenylarsenic dibromide (3313-89-1) was synthesized [32]. Triphenylbismuth dichloride (594-30-9) was purchased from Aldrich Chemical Co., Milwaukee, WI; triphenylantimony dichloride (594-31-0) dichloride was purchased from Strem Chemical Co., Newburyport, MA. Histamine (51-45-6) was obtained from Sigma-Aldrich, Saint Louis, MO.

Physical characterization

Molecular weight was determined in DMSO solution employing light scattering photometry employing a Brice-Phoenix Universal Light Scattering Photometer Model IR results were obtained using a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ¹H NMR spectra were obtained in d-6 DMSO employing Varian Inova 400 MHz and Varian 500 MHz spectrometers.

A Voyager-DE STR BioSpectrometer, Applied Biosystems (Foster City, CA) was used to obtain high resolution electron impact positive ion matrix assisted laser desorption ionization time of flight, HR MALDI-TOF, mass spectra. An accelerating voltage of 25,000 was used with spectra obtained over a mass range of 500 to 2500 Da. Typically 200 shots were used for each spectra. The matrix material was alpha-cyano-4-hydroxycinnamic acid with the matrix placed in a small glass vial along with copper spheres with shaking resulting in a fine powder that was used as the sample.

Cell testing

The toxicity of the test compound was evaluated for a number of cancer cell lines. Cells were placed into a 96-well culture plate at a density of 20,000 cells per 100 μ L of culture medium. Following a 24 hr. incubation period, The test compounds were incubated for 24 hours at concentrations ranging from 0.0032 to 32,000 ng/ml and allowed to incubate at 37°C with 5% CO₂ for 72 h. Titer-Blue reagent (Promega Corporation) was added (20 μ l/well) and the cells incubated for an additional 2 h. Fluorescence was determined at 530/590 nm and converted to % cell viability versus control cells.

To ensure that toxicity was due to the compounds and not diluents, "mock-treatments" were carried out where the same procedure was carried out except omitting the test compound. This mock-treatment never resulted in an inhibition of greater than 1%.

Results and Discussion

Yield and chain length

Table 1 represents yield and chain length of products formed during the reaction of the histamine and Group 15 triphenylmetallic dihalides.

Table 1. Polymer product yield molecular weight and chain length as a function of Lewis Base for the reaction between the various Lewis bases and histamine. The particular reaction concentrations and conditions are given the experimental section.

Lewis Base	% Initial Yield	Additional % Yield/2 Days	Overall % Yield	Molecular Weight	DP
Ph ₃ AsBr ₂	20	4	24	2.4 × 10 ⁶	5800
Ph ₃ SbCl ₂	10	8	18	7.4 × 10 ⁴	160
Ph ₃ BiCl ₂	13	11	24	4.5 × 10 ⁶	8200

Product is formed in thirty second and less. Yield is in the low range. For polymerizations employing Group 15 triphenylmetallic halides, additional product precipitates from the reaction system after the initial product precipitates from the reaction solution [6,10-19]. For the histamine polymers, the arsenic and bismuth products have high molecular weights while the antimony has only a moderate chain length. It is difficult to describe synthetic trends because two different organic solvents were employed with chloroform used for the antimony and arsenic as the organic liquid while the bismuth system used carbon tetrachloride as the organic liquid since triphenylbismuth dichloride is soluble in carbon tetrachloride but not in chloroform. Triphenylarsenic dibromide is brown because of the presence of the As-Ph brown color site. The bismuth and antimony products are white because of the absence of a color site in the reactants. The products showed good adhesion to the glass Petri dish.

Infrared spectroscopy

Infrared spectra were taken of the monomers and polymers. Results for the arsenic and antimony polymers appear in **Table 2**. The band at 2968 (all bands are given in cm⁻¹) characteristic of the ring NH stretch in histamine is absent in the spectra of the polymers. Further, the band at 1596 due to NH₂ scissoring found in histamine is absent in the spectra of the polymers. Both of these are consistent with the formation of the M-N linkage. The NH ring stretch should be missing because it is no longer present being replaced by the M-N moiety. The aliphatic NH₂ is now a NH-M so scissoring is no longer possible. The presence of a new band about 1250 is consistent with the formation of the M-N linkage. Further, bands characteristic of both the histamine and triphenylmetal moiety are present. Band assignments are consistent with reports for other studies [6,10-19,33-37]. Thus,

infrared spectroscopy evidence is consistent with the proposed polymer repeat unit.

Proton NMR

NMR was carried out on the products refer **Table 3**. All values are given in ppm. Assigned bands correspond to the numbers given in the **Figure 3**. Expected bands are present for both the two monomers and resulting polymers. Assignments are consistent with those reported in literature.

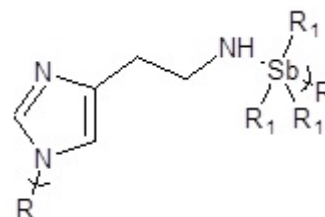


Figure 3. General repeat unit for the polymeric product from reaction of triphenylantimony dichloride and histamine where R1 represents phenyl substituents on antimony and R simple chain extension.

Table 3. NMR bands found for the monomers histamine, triphenylarsenic dibromide and triphenylantimony dichloride and the polymeric products from triphenylarsenic dibromide and triphenylantimony dichloride with histamine. Assignment band numbers are given in the **Figure 3** for histamine.

Assignment	Histamine	Ph ₃ AsBr ₂	Polymer	Ph ₃ SbBr ₂	Polymer
C-H Ring, Ortho		8.2	8.16	8.2	8.2
C-H Ring, Meta, Para		7.70, 7.60	7.71, 7.60	7.70, 7.60	7.68, 7.49
1	11.6				
5	7.4		6.9		7.38
6	3.3		3.3		3.12
7	2.99		2.99		3.01
8	1.3		2.48		2.48

The band associated with the ring N-H is missing since it is now N-M. The band associated with the amine is shifted as expected from about 1.3 to about 2.5.

MALDI MS analysis

MALDI MS was developed to investigate non-volatile solids [38-41] because the polymers are not soluble in suitable volatile liquids to allow intimate association between the matrix and polymer sample traditional MALDI MS is not possible. We have been investigating the use of an alternative approach that focuses on the fragments [14-19]. This technique has been

reviewed [42-47]. MALDI analysis was performed on the polymers.

Table 2. Assigned infrared spectral peaks found in the spectra for the monomers histamine, triphenylarsenic dibromide, and triphenylantimony dichloride and the polymer products of triphenylarsenic dibromide and triphenylantimony dichloride with histamine. Band locations are given in cm^{-1} .

Assignment	Ph_3AsBr_2	Histamine	Polymer	Ph_3SbCl_2	Polymer
CH ip st	3064		3051		3083
CH st		3083	3083	3060	3048
CH_2 asym st		2984	2991		2994
N-H ring st		2968			
CH_2 sym st		28,922,810	#####		28,962,850
ring st		162,515,251,474	#####		162,615,301,478
NH_2 scissoring		1596			
C=C st	1465		1461	1465	1478
M-Ph st	1438		1438	1440	1432
C=C ip wag	1362		1360	1360	1359
CH_2 wag		1346	1346		1332
CH_2 twist		1294	1295		1305
M-N			1231		1247
C-C st	11,781,152		#####	11,781,152	11,821,155
M-Ph sym st	1050		1053	1048	1068
CH ip bend		1090	1086		1094
Sket st		1030	1023		1021
Ring vib	997		997	997	997
Ring breath		960	950		962
lp ring		851	860		862
Skeletal op wag		781	794		805
Sym op bend	736		741	732	725
Aryl op bend*	690		690	690	687

*Asymmetric out-of-plane bending of ring hydrogens for only mono-, meta-, or 1,3,5 substitutions.

Figure 4 contains a portion of the MALDI MS for the triphenylarsenic polymers and **Table 4** contains assignments for the major ion fragments over the entire range studied (to 1200 Da). All ion fragments are described in terms of Da.

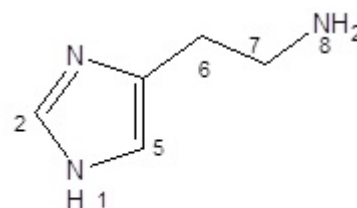


Figure 4. NMR band locations associated with the histamine moiety corresponding to NMR bands given in **Table 4**.

Abbreviations are used to describe the assignments. These are U for unit, 2U for two repeat units, N for nitrogen, HA, for histamine minus two protons, Na for sodium and Ph for phenyl. These abbreviations are also employed for assignments for the other polymers. Loss of phenyl groups occurs. This is common because of the sensitivity of alkyl-metal linkages to the radiation employed in MALDI MS. This loss is believed to occur only at sites of chain scission. The mildness of MALDI MS is shown by the lack of fragmentation of histamine.

Table 4. Ion fragment clusters derived from the product of triphenylarsenic dibromide and histamine.

Ion Frag., Da	(Tentative) Assignment	Ion Frag., Da	(Tentative) Assignment
542	U+HA,Na	758	2U-Ph
589	U+PhAs, Na	781	2U+N-Ph
645	U+Ph ₂ As	846	2U+NH
668	U+Ph ₂ As,Na	871	2U,NH,Na
721	U+Ph ₃ As	1046	2U+Ph ₂ Sb-NH
736	U+Ph ₃ As,N		

Ion fragment clusters derived from the product of triphenylantimony dichloride and histamine are given in **Table 5**. Fragments to over three units are found.

Table 5. Major ion fragment clusters derived from the MALDI MS for triphenylantimony dichloride and histamine polymer product obtained over the mass range of 500 to 2000 Da. They are described as ion clusters because they contain ions from various antimony isotopes.

Ion Frag. Cluster, Da	(Tentative) Assignment	Ion Frag. Cluster, Da	(Tentative) Assignment
540	U+HA-2NH	776	2U+PhSb
575	U+HA	890	2U+NH,Na-Ph
621	2U,NH,Na-2Ph	1106	2U+PhSb
656	U+PhSb	1370	3U-NH
736	U+Ph ₂ Sb	1490	3U+HA

Unlike bismuth and arsenic which have no natural occurring isotopes, antimony contains two isotopes. This allows isotopic matches to be made. **Table 6** contains matches for ion fragment clusters containing one, two and three antimony atoms. The agreement is reasonable to that shown as "Known", appearing in the two most left-hand columns and is consistent with the present of one, two, and three antimony atoms within these ion fragment clusters. The right hand columns contain the particular ion fragment location (Da) and the percentage relative

abundance. The entry above this is the proposed structure for the particular ion fragment cluster.

Table 6. Isotopic abundance matches for antimony-containing ion fragments derived from the MALDI MS of the product of triphenylantimony dichloride and histamine where the ion fragments contain one (top), two (center) and three (bottom) antimony atoms in the observed ion fragment. The first column gives the known mass (Da) for antimony followed by the percentage relative abundance for that particular isotope. The second two columns contain the found mass for the particular ion fragment whose structural assignment is given followed by the experimentally found relative abundance mass associated with that antimony-containing ion fragment.

Known for Sb		U+PA-CO ₂ O	
121	100	608	100
123	75	610	74
Known for 2Sb		2U+Na, NH-2Ph	
242	67	888	68
244	100	890	100
246	37	892	39
Known for 3Sb		2U+PhSb	
363	45	1104	52
365	100	1106	100
367	75	1108	71
369	19	1110	19

Table 7 contains the ion fragment assignments for the product from triphenyl bismuth dichloride and histamine. Ion fragments to over three units are found.

Table 7. Major ion fragments derived from the MALDI MS of the product of triphenylbismuth dichloride and histamine over the approximate range of 500 to 2000 Da. The first column contains the mass of the particular ion fragment and the second column contains the tentative structural assignment for that ion fragment.

Ion Frag. Da	(Tentative) Assignment	Ion Frag., Da	(Tentative) Assignment
532	U-NH	935	U+Ph ₂ Bi, Na
550	U	1083	2U-NH
627	U+HA-2NH	1156	2U+HA,Na-Ph
658	U+HA	1207	2U+HA
681	U+HA,Na	1230	2U+HA,Na
835	U+PhBi	1429	3U+HA
912	U+Ph ₂ Bi		

As in other studies, chain scission occurs primarily at the heteroatoms as shown in **Figures 5 and 6**.

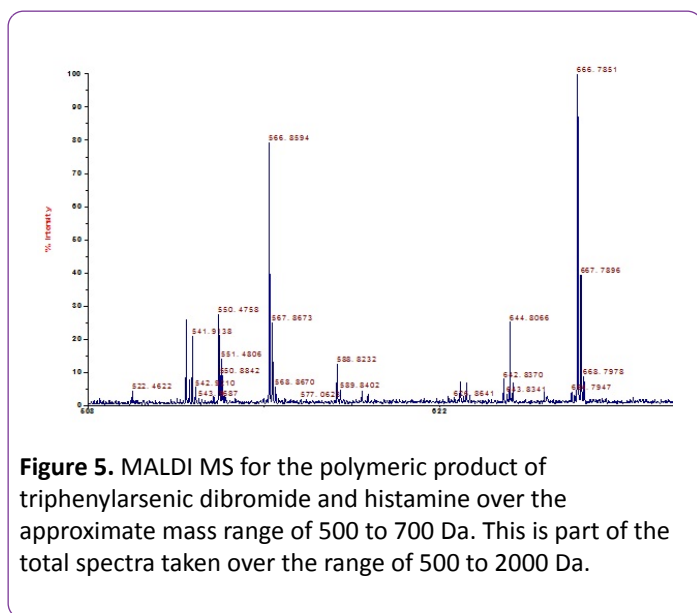


Figure 5. MALDI MS for the polymeric product of triphenylarsenic dibromide and histamine over the approximate mass range of 500 to 700 Da. This is part of the total spectra taken over the range of 500 to 2000 Da.

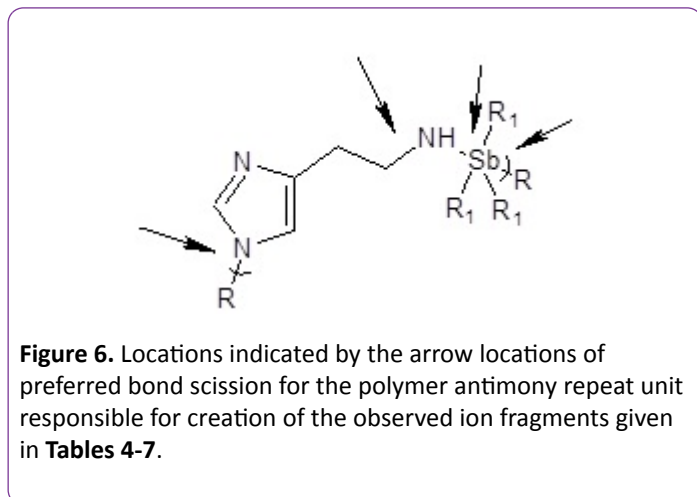


Figure 6. Locations indicated by the arrow locations of preferred bond scission for the polymer antimony repeat unit responsible for creation of the observed ion fragments given in Tables 4-7.

Tumor analysis

Cancer is the leading cause of death world-wide. In this study we used a broad range of solid cancer cell lines (Table 8).

The polymer drugs are cytotoxic and cell death is by necrosis [45,48,49]. Anticancer activity is occurs with the intact polymer without polymer degradation [45,49,50]. This is in agreement with our observations that the polymers do not degrade in DMSO with half-chain lives, the time for the polymer chain length to halve, greater than 30 weeks [45,48,49].

Most organometallic compounds associate with polar solvents such as DMSO. This association can influence the observed biological results. We find that this is generally not the case [45-54] for polymers with similar structure to those reported here with the influence less than 20% [45,50].

Evaluation of the effectiveness for test material to inhibit cell growth is generally found using two measures. The first measures the amount required to reduce growth of a particular cell line. This concentration is often called effective concentration, EC. Thus the concentration of the test material inducing a response halfway between the baseline and

maximum after a specified exposure time is called the 50% response concentration, EC_{50} .

Table 8. Descriptions for the cell lines employed in the current study.

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

The two most widely used cell line standards are the NIH/3T3 (often referred to as simply 3T3 cells) and WI-38 cell lines. NIH/3T3 cells are mouse embryo fibroblast cells that are partially transformed cells. Unlike normal cells they are immortal but retain other normal cell characteristics. They are robust and more easily maintained compared to normal cell lines.

The second standard cell line is WI-38 cells which are normal embryonic human lung fibroblast cells. Compared to NIH/3T3 cells, they are more fragile and difficult to maintain. Typically, they are preferred over the WI-38 cells because of ease of handling aided by an infinite life span.

In this study, the EC_{50} values for the NIH/3T3 and WI-38 cell lines give similar values for the cancer cell lines. When there is a difference, it is customary to employ WI-38 cell results [55].

EC_{50} values for the monomers and polymers are given in Table 9. Values for cisplatin are also given. Cisplatin, and related platinum drugs, is among the most widely employed anticancer drugs. It is highly toxic with many unwanted side effects. Many of the tested compounds have low toxicities towards the tested cell lines. Histamine shows no ability to inhibit any of the cell lines to the concentration limits employed.

All of the polymers inhibit one or more cancer. Not inhibiting pancreatic cancer is one of our main focuses because it does not have a "cure" once it metastasizes. In the USA nearly 32,000 individuals die each year from pancreatic cancer. The human prostate cancer cell lines employed in the current study are AsPC-1, an adenocarcinoma pancreatic cell line responsible for about 80% of the human pancreatic cancers and PANC-1an

epithelioid carcinoma pancreatic cell line which that appears in 10% of human pancreatic cancers. The bismuth and antimony show inhibition of both pancreatic cancer cell lines.

Table 9. EC₅₀ Concentrations (micrograms/mL) found for the tested compounds as a function of the particular cell lines whose description is given in **Table 8**.

Sample	WI-38	3T3	AsPC-1	PANC-1
Ph ₃ AsBr ₂	>32000	>32000	390(11)	>32000
Ph ₃ As/HA	>32000	>32000	>32000	10(0.97)
Ph ₃ SbCl ₂	3500(34)	3600(21)	>32000	6500(18)
Ph ₃ Sb/HA	1.12(0.11)	2.48(0.24)	8.5(0.88)	7.9(0.83)
Ph ₃ BiCl ₂	790(90)	600(16)	3700(22)	2100(10)
Ph ₃ Bi/HA	1.7(0.14)	0.27(0.03)	3.9(0.35)	89(9.7)
Histamine(HA)	>32000	>32000	>32000	>32000
Cisplatin	0.015(0.01)	0.0023(0.005)	0.0023(0.005)	0.0035(0.005)
Sample	7233/MDA	7259/MCF-7	1507/HT-29	3465/PC-3
Ph ₃ AsBr ₂	21.(1.0)	24.(2.1)	16.(1.2)	20.(1.7)
Ph ₃ As/HA	>32000	>32000	>32000	>32000
Ph ₃ SbCl ₂	12.4(1.1)	130(11)	33.(3.1)	38(4)
Ph ₃ Sb/HA	2.2(0.26)	21(2.7)	1.5(0.22)	3.8(0.29)
Ph ₃ BiCl ₂	1.4(0.2)	1.6(0.21)	2.2(0.16)	2.4(0.16)
Ph ₃ Bi/HA	0.32(0.03)	3.2(0.29)	4.7(0.41)	4.1(0.42)
HA	>32000	>32000	>32000	>32000
Cisplatin	0.0044(0.004)	0.0029(0.002)	0.0041(0.003)	0.0057(0.003)

Note: Values given in () are standard deviations for each set of measurements.

The breast cancer cell lines 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 there existed a major difference in the polymer's ability to inhibit the two breast cell lines [45,48,55-57]. When the Lewis base in the polymer possessed a O-Phenylene group such as found in some hormone treatments, the polymer showed greater ability to inhibit the MDA-MB-231 estrogen-independent cells possibility because the MCF-7 cells reacted with the polymers removing them from inhibiting the MCF-7 cells. Here, the arsenic product shows little ability to inhibit either breast cell line, but both the antimony and bismuth show good ability to inhibit both cell lines, favoring the inhibition of the estrogen independent MDA-MB-231 cell line at about a

tenfold lower concentration compared to the estrogen receptor positive MCF-7 cell line. Histamine does not possess an O-Phenylene structure so it is currently not known why this difference exists.

Both of the antimony and bismuth polymers show good ability to inhibit the 1507 colon and 3465 prostate cell lines. Colorectal cancer is also referred to by other names such as rectal cancer, colon cancer, colorectal adenocarcinoma, and bowel cancer. The focus is on treating uncontrolled cell growth, cancer, in the colon or rectum or related area of the body. These various cancers are genetically the same cancer. Cancers confined within the colon wall are generally curable with surgery while cancer that has spread throughout the body is typically not curable and management is by chemotherapy and improving the quality of life. Colorectal cancer is the third most diagnosed cancer worldwide being most common in developed countries. According to the American Cancer Society for 2014 about 137,000 people will be diagnosed with colorectal cancer with about 50,000 predicted to die of the disease in the USA. The HT-29 cell line is the most widely employed colon cancer cell line for studying a compounds ability to inhibit cell growth.

Based on EC₅₀ results, the antimony and bismuth polymers show a good ability to inhibit most of the cancer cell lines while the arsenic polymer exhibits little ability to inhibit any of the cancer cell lines.

The second measure, often called the chemotherapeutic index, CI, of the potential use of compounds as anticancer materials is the concentration of drug necessary to inhibit the standard cells compared to the concentration of drug necessary to inhibit the growth of the test standard cell line, here WI-38 and NIH/3T3 cells. Here the focus is on the WI-38 results since these are preferred compared to the NIH/3T3 results. The CI₅₀ is simply the ratio of the EC₅₀ for the standard cell line WI-38 cells divided by the EC₅₀ for the particular test cell.

CI₅₀ values derived from values given in **Table 9** are given in **Table 10**. (Please note that the experimental concentration upper limit is 32000 nanograms/mL is found in some cases. When samples exceed this limit they are cited as >32000 and values of >32000 divided by some value are entered as > followed by a number.

CI₅₀ values of two and greater are generally considered significant. There is a single instance where a CI₅₀ value is greater than 2. This is the large CI₅₀ value >3200 found for the arsenic polymer against the PANC-1 pancreatic cancer cell line. It is a result of the polymer having a very low toxicity towards the standard WI-38 cell line coupled with a decent toxicity towards the PANC-1 pancreatic cancer cell lines. This high CI₅₀ value is significant with further testing signaled for this polymer. In all cases, once inhibition begins, there is a steep slope in the inhibition verses concentration curve with total inhibition the end result.

Table 10. CI₅₀ values calculated from the EC₅₀ data given in **Table 9** as simple ratios of the EC₅₀ for the standard value obtained from the standard WI-38 cell line divided by the observed EC₅₀ for the particular cancer cell line type. The samples are all polymeric with the exception of cisplatin.

Sample	EC ₅₀ WI-38/	EC ₅₀ WI-38/	EC ₅₀ WI-38/	EC ₅₀ WI-38/	EC ₅₀ WI-38/	EC ₅₀ WI-38/
	EC ₅₀ AsPC-1	EC ₅₀ PANC-1	EC ₅₀ HT-29	EC ₅₀ PC-3	EC ₅₀ MCF	EC ₅₀ MDA
Ph ₃ As/HA	1	>3200	1	1	1	1
Ph ₃ Sb/HA	0.13	0.14	0.73	0.29	0.052	0.5
Ph ₃ Bi/HA	0.43	0.019	0.36	0.41	0.53	5.3
Cisplatin	8.2	5.4	4.3	6.6	4	3.3

Summary

Group VA polyamines have been synthesized in low yield with moderate to high molecular weight employing commercially available reactants, with the exception of the triphenylarsenic dibromide. The interfacial polycondensation is employed industrially to synthesize aramid fibers and polycarbonates [58,59]. Thus, there is a straightforward pathway production of the polymers from small to large amounts. Infrared spectroscopy shows the formation of new bands characteristic of the formation of M-N bonds. MALDI MS shows formation of ion fragment clusters about three units long with isotopic abundance results for the antimony polymers showing the presence of antimony in the ion fragment clusters. The absence of the histamine ring proton in the NMR spectra is consistent with the formation of the polyamine structure. Tumor analysis shows that the arsenic and bismuth polymers exhibit good cancer cell inhibition of all of the tested cancer cell lines. The arsenic polymer shows among the best CI₅₀ values thus far found against the PANC-1 pancreatic cancer cell line. It will be considered for live animal testing.

This study is part of an ongoing effort whereby various Lewis acids and bases are combined in an effort to more fully understand which combinations are best at inhibiting cancer cell lines. While pancreatic and breast cancers are particular focal cancer, all of the cancers are of concern. Until a few years ago, there were no good agents that could inhibit pancreatic cancer cell lines so such studies give us and other researchers added evidence with respect to which compounds might be important to follow up with live animal studies. The current study gives one such combination worthy of additional study.

Histamine is one of the initial diamine Lewis bases studied by us and its ability to show some inhibition of all of the cell lines indicates that further work with diamine-containing reactants is warranted.

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