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SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF GROUP 4 METALLOCENE POLY (AMINE ESTERS) FROM 6-AMINOPENICILLANIC ACID AND THEIR AIBLITY TO INHIBIT SOLID TUMOR CANCER CELL GROWTH

Charles E. Carraher, Jr.[1]*, Michael R. Roner[2], Dhruivin Patel[1], Alisa Moric-Johnson[2], Lindsey Miller[2], Paul Slawek[1], Francesca Mosca[1], Jessica Frank[1] and Loretta Chen[1]

[1]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431, USA.
[2]University of Texas Arlington, Department of Biology, Arlington, TX 76010 USA.

***Corresponding Author: Charles E. Carraher, Jr.**

Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431, USA.

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ABSTRACT

Poly (amine esters) were synthesized employing interfacial polycondensation of the salt of 6-amiopenicillanic acid and group 4 metallocene dichlorides in decent yield and high chain lengths. Infrared spectroscopy shows bands characteristic of the expected N-M and C(C=O)-O-M linkages with the backbone consisting of largely asymmetrical units present in a non-bridged structure. MALDI MS shows ion fragments to 7 and 8 units long with good isotopic abundance agreements. The polymers inhibit the growth of all of the human cancer cell lines including two breast and two pancreatic cancers.

KEYWORDS: Group 4 metallocene poly (amine esters), 6-aminopenicillanic acid, pancreatic cancer, breast cancer, MALDI MS, interfacial polycondensation.

INTRODUCTION

We have been involved in the inclusion of metal-containing moieties within polymers for various reasons.[1-3] Recently, the focus is on developing drugs to combat harmful pathogens and infectious agents involved with viruses, bacteria, and cancer. Overall, much of our relational involves coupling of the metal-containing moiety, typically as a Lewis acid, with a Lewis base that itself is biologically active, but not necessarily related to cancer, hoping for a synergetic effect. Here the focus is on cancer, specifically pancreatic cancer employing Group 4 metallocenes. Two metal-containing agents have undergone human testing. The first contains platinum specifically cisplatin and related drugs.[4] The second is titanocene dichloride that has undergone Phase I and II clinical trials.[5-9] While the trials were generally positive, it showed a number of undesirable biological drawbacks including inducing nausea, hypoglycemia, leaving a metallic taste, etc. Overall, titanocene drugs exhibit poor solubility. In a related study, we recently developed water soluble titanocene-containing polymers based on incorporation of poly(ethylene glycol) units.[10]

The Lewis base for the present study is 6-aminopenicillanic acid, 6-APA. Its Chemical Abstract, CA, name is 6-amino-3,3-dimethyl-7-oxo-,(2S,5R,6R) 4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. We will refer to it as simply 6-APA or APA. 6-APA serves as a core starting material for a number of penicillin's. Initially it was derived from the fermentation of Penicillum mold and initially discovered in 1958.[11]

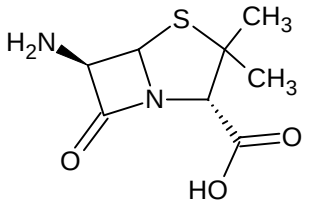


Fig. 01: Structure of 6-APA.

Reports have described the incorporation of 6-APA into polymers often for the purpose of controlled release of 6-APA. The reaction between gellan and 6-APA allowed the release of the 6-APA.[12-16] Optically active polymers were formed from the reaction between 6-aminopenicillanic acid benzyl ester p-toluenesulfonate salt, which was subsequently reacted with acryloyl chloride yielding 6-(acryloylamino)penicillanic acid benzyl ester. This was polymerized giving the desired polymer incorporating the 6-APA moiety.[17]

Copolymers of N-(2-hydroxypropyl)methacrylamide) containing p-nitrophenyl esters of N-methacryloylated oligopeptides and N-methacryloylamino-phenoxyacetic acid were employed to bind 6-APA as a side-chain unit.[18] Penicillin acylase was bound to water-soluble

carriers such as dextran and starch, producing 6-APA products.[18,19] Finally, oligomeric chains were formed from the ring opening of 6-APA.[20]

This paper describes the synthesis of polyamine esters (Figure 2) from the reaction of group 4 metallocene dichlorides with the salt of 6-APA and their ability to inhibit the growth of various solid human cancer cell lines including pancreatic cancer cell lines.

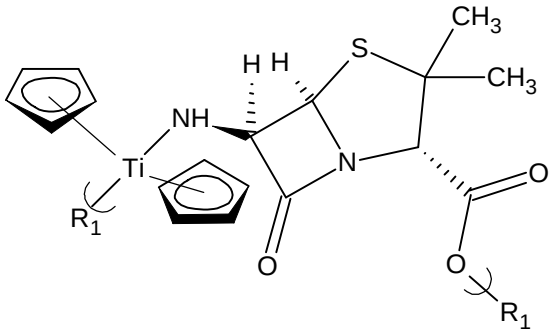


Fig 02: Repeat unit of the salt of 6-APA with group 4 metallocene dichlorides, here titanium, where R represents simple chain extension.

EXPERIMENTAL

The interfacial polycondensation technique was employed to synthesize the polymers. Initially, 6-APA (0.00300 mol) and sodium hydroxide (0.0060 mol) dissolved in water (30 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 ^{o}C). Stirring (about 18,000 rpm) was begun and a chloroform solution (30 ml) containing the metallocene dichloride (0.00300mol) added (about 3-4 seconds) through a hole in the jar lid using a powder funnel. Blending continued for 15 seconds. The precipitate was recovered using vacuum filtration and washed several times with deionized water and chloroform to remove unreacted materials and unwanted by-products. The solid was washed onto a glass Petri dish and allowed to dry at room temperature.

Titanocene dichloride (1271-19-8), zirconocene dichloride (129-32-3), hafnocene dichloride (12116-66-4) and 6-aminopenicillanic acid ((551-16-6) were purchased from Aldrich Chemical Co., Milwaukee, WS and used as received.

Molecular weight was determined in dimethyl sulfoxide, DMSO, solution employing light scattering photometry employing A Brice-Phoenix Universal Light Scattering Photometer Model 4000 was employed to obtain molecular weight on dimethyl sulfoxide solutions containing dissolved polymer. A JASCO FT/IR-4100 fitted with an ATR Pro 450-s was used to obtain the attenuated total reflectance infrared spectra.

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 5000 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 power that was used as the sample.

The toxicity of each 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 µL of culture medium. Following a 24 h 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% CO_2 for 72 h. Titer-Blue reagent (Promega Corporation) was added (20ul/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%. Once inhibition began, the curve of the inhibition verses concentration plot was steep ending at 100% inhibition.

RESULTS AND DISCUSSIION

Yield and chain length

Table 1 contains the percentage yield and chain length for the metallocene/6-APA products. Yield is modest and all of the products are polymeric. There is no explanation for the observed trend. The interfacial system is complex and dependant on a number of factors including monomer and polymer solubility in the various phases and overall reaction.

Table 01: Product yield, average molecular weight, and chain length for the metallocene poly (amine esters).

Metallocene	Percentage Yield	Molecular Weight	Chain Length, DP
Cp_2Ti	25	2.3×10^6	5800
Cp_2Zr	61	4.3×10^5	990
Cp_2Hf	15	4.5×10^5	860

Infrared Vibration Spectroscopy

Infrared spectra were obtained for the monomers and polymers. Table 2 contains results for selected bands.

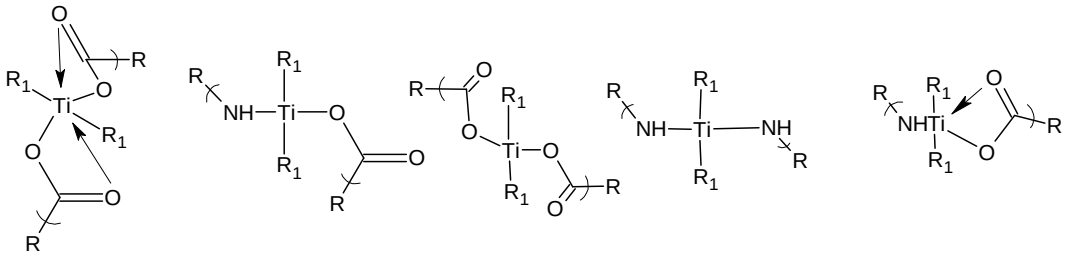
Table 02: Infrared spectral bands for titanocene dichloride, hafnocene dichloride, 8-APA and the resulting 6-aminopenicillanic acid-metallocene polymers. (Frequencies are given in wavenumbers.)

Band Assignment	Cp_2TiCl_2	6-APA	Cp_2Ti/6-APA	Cp_2HfCl_2	Cp_2Hf/6-APA
O-H st		3678			
NH_2 st		3670,3651			
CH-st Arom	3103		3107	3115	3108
CH st		2960,2927	2963,2927		2955,2923
CH asy St		2872,2858	2871,2855		2870,2853
C=O (internal)		1774	1767		1749
C=O		1624	1627		1630
CH_3sym st		1463	1456		1455
Cp st	1440		1437		1444
CH wag: C=C,C-C st	1370		1369		1367
CH bending		1339	1337		1337
M-O asy st		1309			1309
C-NH		1256	1258		1255
M-N			1161		1158
M-)-C=O st			1091		1093
CH ip wag	1014		1017		1018
CCN asym st		962	960		964
CC wag		905	924		922
CH ip wag	873,827		851,827		890,815
M-O sym st			725		750

Bands are present from both the metallocene units and 6-APA. Bands present in the 6-APA associated with the OH and NH_2 are absent in the polymers consistent with their replacement through reaction with the metallocene dichlorides. Other bands derived from both the metallocene and 6-APA are found consistent with their presence in the polymer backbone.

New bands characteristic of the formation of the metallocene linkage to the Lewis base are present. The M-N band appears at about 1160. Several M-O bands are possible including those from attachment to the R-C(O)-O moieties. These bands can be associated with various stretching, torsonal, etc. These appear as new bands for the polymer characteristic for the M-O asymmetric stretch about 1310. The M-O symmetric stretch is assigned in the region between 725 to 750. A new band about 1090 is assigned to the M-O-C(O) stretch. Thus, bands are present characteristic of the formation of the M-O-C(O) and M-N linkages.

There are five possible linkages about the metallocene metal (Figure 3). Three involve the amine group, Figure 3b, 3d and 3e. There are also two different geometries are possible for the carboxylic arrangement (Figure 3a, 3b, 3c and 3d). One of these is described as a distorted four-bonded "bridging" structure shown in Figure 3a and 3e, where the carbonyl back-bonds on the metal forming "bridged" connections to the metal atom. The corresponding "non-bridged" structure is given in Figures 3b and 3c. These arrangements are also referred to as simply bridging and linear or non-bridging structures.



Where a is symmetrical diacid, bridged; b is amine, non-bridged acid, c is symmetrical non-bridged diacid, d is symmetrical diamine, and e is amine and bridged acid

Fig 03: Possible geometrical arrangements about the titanium atom.

Infrared spectroscopy is the easiest way to determine the presence of bridged and non-bridging.[2,3,21-23] Bridging asymmetric carbonyl absorptions are found around 1550-1590. The bridging symmetric carbonyl band is found around 1390-1425. Non-bridging asymmetric carbonyl bands are found about 1600-1650; and the corresponding symmetric carbonyl bands are found about 1350-1390. Results for the products are given in Table 3.

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

Metallocene Moiety	Asym Non-bridging	Sym Non-bridging	Asym Bridging	Sym Bridging
Cp_2Ti	1627(l)	1385(l)	1572(s)	----
Cp_2Zr	1609(l)	1392(l)	1573(s)	1417(s)
Cp_2Hf	1610(m)	1387(l)	1583(m)	1417(m)

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

For the titanocene and zirconocene products, bands associated with the non-bridging are large while those associated with bridging are small or absent. This is consistent with the products being largely of the non-bridging arrangement. All four bands are present for the hafnocene product consistent with the product having a combination of both types consistent with the product having both types of bonding about the metallocene. Further, we find that bridging typically occurs when there are two carboxyl groups on the same metal atom so that the products largely exist within the polymer backbone as having mainly asymmetrical structures rather than symmetrical structures (Figure 4). Thus, the polymer chains contain largely alternating repeat units within the polymer chain.

(CO)O-M-O(CO)	N-M-N	N-M-O(CO)
Symmetrical	**Symmetrical**	**Asymmetrical (Mixed)**

Fig 04: Possible repeat unit structures about the metallocene atom within the polymer chain focusing on the arrangement of the 6-APA.

MALDI MS

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 investigated the use of an alternative approach that focuses on the fragments. This technique has been reviewed.[24-26] MALDI MS analysis was performed on the polymers. Two general MALDI MS modes were employed. These are 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.

Figure 5 contains the MALDI MS for the linear mode for the mass range of 700 to 1,000 Da. Table 4 contains the results for the titanocene polymer. Several abbreviations are employed in describing the assigned ion fragment clusters-U = one unit, 2U=2 units; APA for 6-aminopicillanic acid minus two protons; Na for sodium, a common contaminant.

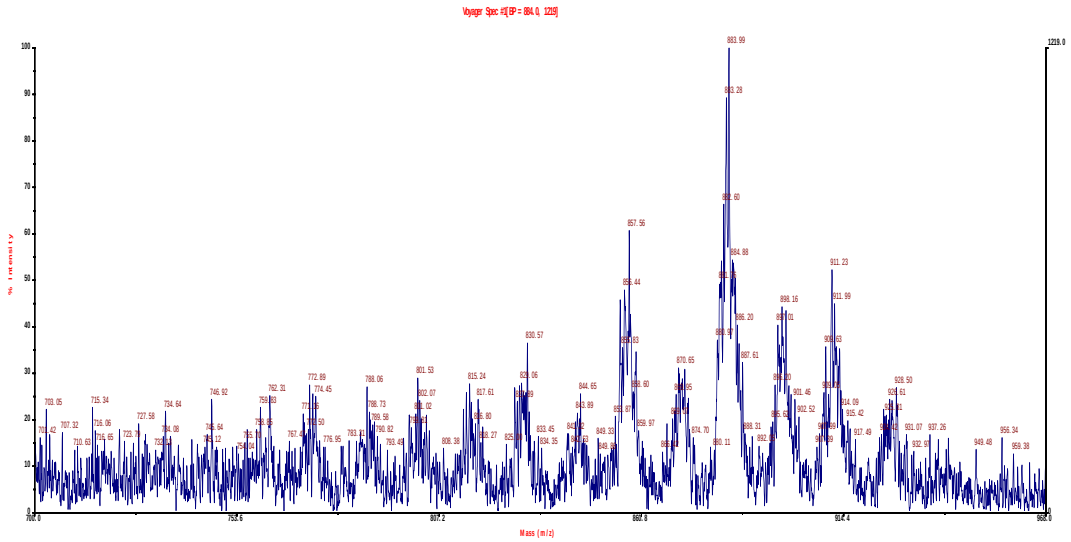


Fig 05: MALDI MS for the product of titanocene dichloride and 6-APA over the approximate mass range of 700 to 1000 Da for the linear mode.

Table 04: Major ion fragment clusters for the product of titanocene dichloride and 6-APA.

Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment	Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment
	619	U+APA,O	1546		4U-O
	627	U+APA,Na		1702	4U +APA-2CO_2
	774	2U-O	2135		5U+APA-CO_2

	802	2U+O		2206	5U+ Cp_2Ti,CO_2
858	859	2U+CO_2,Na		2241	5U+ $Cp_2Ti,2CO_2$,Na
884	885	2U+CO_2,O,Na		2304	6U-CO_2
	925	2U+APA,Na-2CO_2		2373	6U+O
1036		2U+ Cp_2Ti,CO_2,Na		2391	6U+CO_2
	1138	3U-CO_2	2548		6U+APA-O
1155		3U-O	2798		7U+CO_2
1269		3U+2CO_2	3032		7U+ $Cp_2Ti,2CO_2$,Na
	1294	3U+2CO_2,Na	3182		8U+CO_2
1316		3U+APA-2CO_2	3288		8U+APA, Na-2CO_2
	1414	3U+ Cp_2Ti,CO_2,Na	3404		8U+ $Cp_2Ti,2CO_2$
	1503	4U-Cp			

Ion fragment clusters to eight units are found.

Many metals contain isotopes that create different ion fragments for each specific ion fragment containing them with the relative abundance of the ion fragments dependent on the natural abundance of each isotope. This creates "fingerprints" characteristic of this natural isotopic abundance. These fingerprints can often be seen in the MALDI MS such as Figure 5 and can be described in tabular form. Table 5 contains the results for several ion fragment clusters present in Figure 5 containing one and two titanium ions per ion fragment cluster. The matches are reasonable consistent with the presence of one and two titanium atoms within the ion fragment clusters.

Table 05: Isotope abundance matches for ion fragments containing one and two titanium atoms. The first set contains results for ion fragment clusters containing one titanium atom per ion fragment cluster; the second set contains results for two titanium atoms per ion fragment cluster. The first two columns contains the known, natural abundance relative percentage for titanium with column being the mass and column 2 the relative abundance for that mass assignment; columns 3 and 5 contain the ion fragment mass assigned for the structures given above and columns 4 and 6 contain the experimentally found relative abundance for that particular mass.

Known for 1 Ti		**U+APA,O**		**U+APA,Na**	
46	11	617	12	625	11
47	10	618	10	626	11
48	100	619	100	627	100
49	8	620	9	628	8
50	7	621	8	629	8

Known for 2 Ti		**2U-O**		**2U+O**	
94	22	776	22	800	22
95	21	775	20	801	20
96	100	774	100	802	100
97	16	775	17	803	16
98	15	776	16	804	15

Figure 6 contains a portion of the MALDI MS for the zirconocene dichloride product with 6-aminopenicillanic acid and Table 6 contains the major ion fragments and tentative structural assignments for the ions obtained to 3500 Da. Again, abbreviations are employed in describing the proposed structures for the ion fragment clusters similar to those given for the titanocene product.

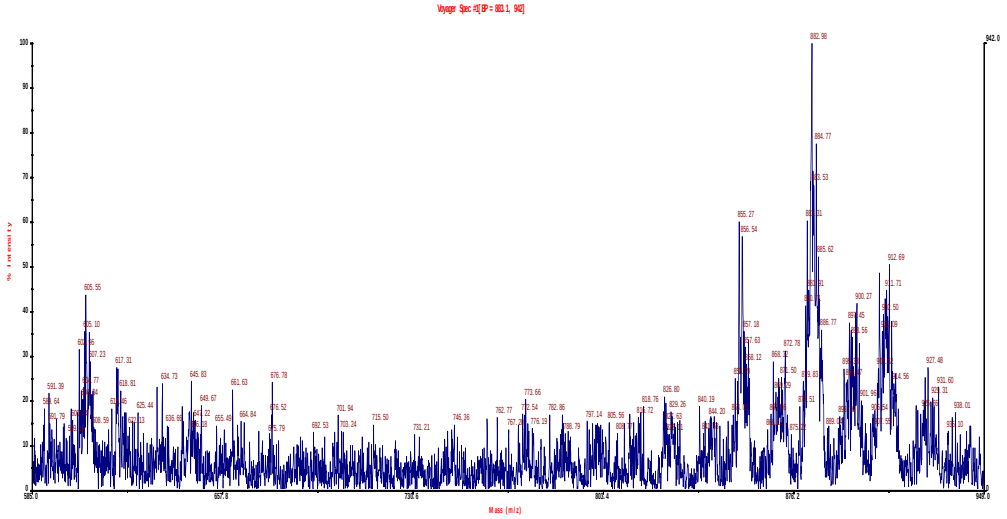


Figure 6: MALDI MS for the product of zirconocene dichloride and 6-APA using the linear mode over the mass range of 700 to 1000 Da.

Table 06: Major ion fragment clusters found for the product of zirconocene dichloride and 6-APA for the linear and reflective mode.

Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment	Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment
	591	U+APA-CO_2,O	1619		3U+ Cp_2Zr,2CO_2
605		U+APA-CO_2		1629	3U+ Cp_2Zr,2CO_2,Na
645	647	U+APA	1730	1732	4U
	665	U+ Cp_2Zr,O	1911		4U+APA-CO_2
826	827	2U-CO_2		1991	4U+ Cp_2Zr,2O
882	883	2U+O		2048	4U+ Cp_2Zr,2CO_2
909	909	2U+O,Na		2219	5U+CO_2
915	914	2U+CO_2		2309	5U+APA-2CO_2
	1016	2U+APA-CO_2,O	2415	2415	5U+ Cp_2Zr,Na
1069		2U+APA-O		2425	5U+ Cp_2Zr,2O
	1090	2U+ Cp_2Zr	2406		6U
	1261	2U-CO_2	2779		6U+APA-CO_2
1317		3U+O	2880		6U+ Cp_2Zr,CO_2,O
	1349	3U+CO_2	2939		6U+ Cp_2Zr,2CO_2,Na
	1457	3U+APA-CO_2,O	3029		7U-O
1491		3U+APA-2O	3212		7U+APA-CO_2
	1527	3U+ Cp_2Zr	3297		7U+APA,CO_2

Ion fragment clusters to seven units are found. Table 7 contains isotope abundance matches for ion fragment clusters containing one and two zirconium atoms. The matches are reasonable consistent with the ion fragment clusters containing one and two zirconium ions per cluster.

Table 07: Isotopic abundance matches for ion fragment contains one, top, and two, bottom, zirconium per ion fragment cluster.

Known for 1 Zr		U+APA-CO_2,O		U+APA-CO_2	
90	100	591	605	605	100
91	22	592	21	606	23
92	33	593	34	607	36
94	34	595	34	609	36
96	5	597	6	611	6

Known for 2Zr		2U-CO_2		2U+O	
180	100	827	100	883	100
181	44	828	42	884	42
182	74	829	73	885	75
183	15	830	12	886	16
184	79	831	75	887	72
185	15	832	16	888	14
186	33	833	31	889	34
188	15	835	16	891	15

Figure 7 contains the MALDI MS for the product of hafnocene dichloride and 6-APA, linear mode, over the mass range of 700 to 1000 Da. Table 8 contains the ion fragment clusters for the hafnocene/6-APA product to 3500 Da. Ion fragments to six repeat units are found. Table 9 contains isotope abundance matches for ion fragments containing one and two hafnium atoms. The matches are reasonable consistent with the ion fragments containing one and two hafnium atoms.

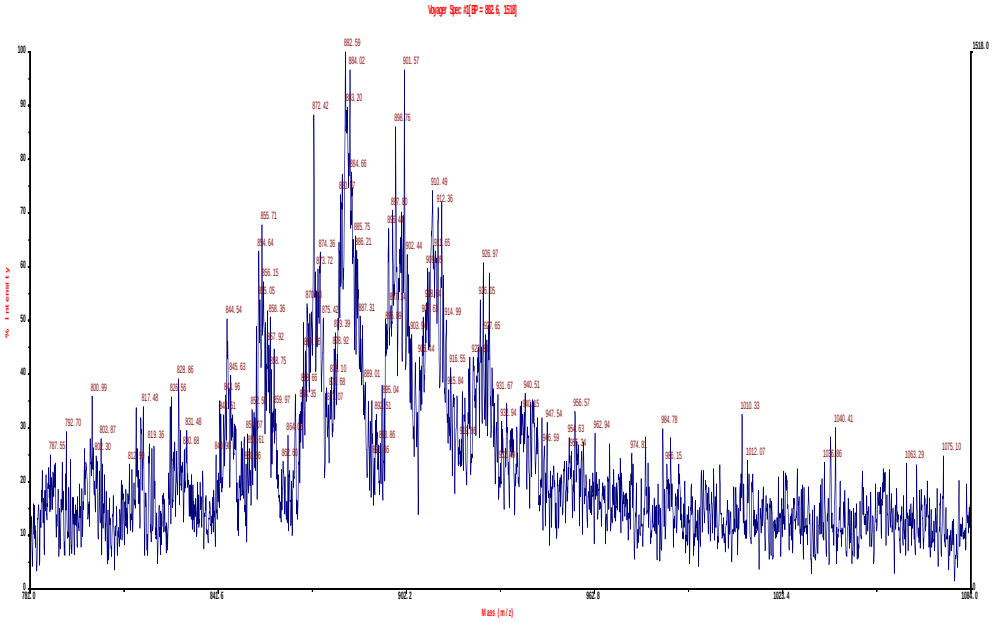


Fig 07: MALDI MS for the product of hafnocene dichloride and 6-APA over the mass range of 700 to 1000 Da for the linear mode.

Table 08: Major ion fragment clusters using the reflective and linear modes for the product of hafnocene dichloride and 6-APA.

Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment	Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment
576	577	U+CO_2		1501	2U+ Cp_2Hf,2CO_2,Na
606		U+CO_2,Na		1559	3U-CO_2
	739	U+AP-O	1645		3U+CO_2
774		U+AP,Na	1803	1803	3U+AP-O
857		U+ Cp_2Hf	2087		4U-CO_2
883	882	U+ Cp_2Hf,2O	2182	2185	4U+CO_2
911		U+ Cp_2Hf,CO_2,O		2262	4U+AP-2CO_2
939		U+ Cp_2Hf,2CO_2		2359	4U+AP
958		U+ Cp_2Hf,2CO_2,Na	2377		4U+AP,Na
1025	1026	2U-CO_2	2480		4U+ Cp_2Hf,Na
	1199	2U+AP-2CO_2	2689		5U+O
1235		2U+AP-CO_2	2748		5U+CO_2,Na
	1277	2U+AP	3152		6U-CO_2
	1422	2U+ Cp_2Hf,CO_2	3236		6U+Na
1477		2U+ Cp_2Hf,2CO_2	3392		6U+AP-2O

Table 09: Isotopic abundance matches for ion fragments containing one, top, and two hafnium, bottom, atoms.

Known for 1 Hf		U+CO_2		U+CO_2,Na	
176	15	572	14	602	14
177	53	573	51	603	53
178	77	574	73	604	75
179	39	575	38	605	38
180	100	576	100	606	100

Known for 2Hf		U+ Cp_2Hf,CO_2,O		U+ $Cp_2Hf,2CO_2$	
353	9	907	10	935	10
354	31	908	30	936	32
355	55	909	53	937	52
356	77	910	78	938	78
357	98	911	100	939	99
358	100	912	99	940	100
359	46	913	45	941	44
360	59	914	58	942	60

As in other studies, ion fragmentation occurs at heteroatoms within the polymer backbone as shown in Figure 8.

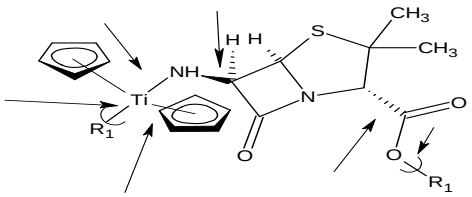


Fig 08: Preferred sites for bond scission for the titanocene/6-APA product.

Cancer Inhibition

Cancer is the leading cause of death globally. The cell lines employed in the current study are given in Table 10. They represent a broad range of important solid tumor cancers.

Table 10: Human 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	Recto-sigmoid colon	Adenocarcinoma
7259	MCF-7	Pleural effusion-breast	Adenocarcinoma
ATCC CCL-75	WI-38	Normal embryonic lung	Fibroblast
	AsPC-1	Pancreatic cells	Adenocarcinoma
	PANC-1	Epithelioid pancreatic cells	Carcinoma

Different measures are typically employed in the evaluation of compounds to control cancer growth. The two most widely employed are used in the present study. The first involves the concentration dose needed to reduce growth of a particular cell line. We will use the term effective concentration, EC, for this. 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}. In other studies, we found that the polymer drugs are cytotoxic and cell death is by necrosis.[2,3] We have recently found that the anticancer activity is brought about by the intact polymer and not through polymer degradation.[2,27] This is consistent with studies that show that polymers are stable in DMSO with half-chain lives, the time for the chain length to halve, generally in excess of 30 weeks.[2,3,28]

Table 11 contains EC_{50} values for the present compounds and monomers. Cisplatin, among the most widely employed chemo-agents, is included as a standard. Consistent with other studies done by us the metallocene monomers are relatively non-toxic.[3,29,30] as is the Lewis base. Even so, the ability of various Group 4 metallocene small molecules to inhibit cancer growth is well established.[2,3,30,33-37] In fact, titanocene dichloride was the first-non-platinum metal-containing compound to undergo clinical trial.[38] The mechanism by which it inhibits cell growth is complex and not fully understood but is believed to be related to the metallocene's ability to interact with the protein transferrin.[38,39]

In the United States about 32,000 individuals are affected with pancreatic cancer. Worldwide this number is about 170,000. Nearly all of those affected with pancreatic cancer die from the ravages of the disease within half a year. It is the fourth leading cause of cancer death worldwide behind lung (1.3 million deaths/year), stomach (1 million deaths/year), and liver (660,000 deaths/year). Pancreatic cancer generally metastasizes prior to detection leading to the poor success rate in its treatment. Further, there is no chemotherapy for metastasized pancreatic cancer. Because pancreatic cancer does not have a generally accepted "cure" our current focus is on the synthesis of materials that might be successful in combating pancreatic cancer. We recently described the ability of a number of organotin polymers to inhibit pancreatic cancer.[2,40-43] More recently, we found that Group VA-containing polymers also exhibit some inhibition of pancreatic cancer cell lines.[44]

The two most widely employed human pancreatic cancer-associated cell lines are employed here. The cell lines tested are AsPC-1 which is an adenocarcinoma pancreatic cell line and PANC-1 which is an epithelioid carcinoma pancreatic cell line. Combined they account for about 90% of the human pancreatic cancers. As seen in Table 12 while the metallocene dihalides themselves do not inhibit pancreatic cancer cell growth the polymers do at concentration levels comparable to cisplatin itself. Further, the EC_{50} values for all three of the metallocene polymers and the two pancreatic cancer cells are almost the same and may signal the ability for these metallocene polymers to inhibit all of the various pancreatic cell lines.

The pair of breast cancer cell lines deserves special comment. They represent a matched pair of cell lines. The MDA-MB-231 (noted in tables as simply MDA; 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 polymers we found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure.[2] In the current study there is little difference in the ability to inhibit the two cell lines by the polymers. The polymers exhibit decent inhibition of both breast cell lines.

The PC-3 (3465) results are of interest because this particular prostate cell line is viewed as the most resistant of the prostate cancer cell lines. All three of the polymers show decent ability to inhibit this cell line. 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 in the appendix. 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. Again, the metallocene polymers exhibit decent inhibit of the HT-29 cell line.

Table 11: EC_{50} values (micrograms/mL) for the tested cell lines for 6-aminopicillinic acid, 6-APA, and the metalocene-containing monomers and polymers. Values given in () are the standard deviations.

Compound	WI-38	PANC-1	AsPC-1
APA	>32	>32	>32
Cp_2TiCl_2	>32	>32	>32
Cp_2Ti/APA	0.71(.5)	0.72(.6)	0.70(.6)
Cp_2ZrCl_2	>32	>32	>32
Cp_2Zr/APA	0.64(.5)	0.70(.6)	0.72(.6)
Cp_2HfCl_2	>32	>32	>32
Cp_2Hf/APA	0.65(.5)	0.69(.6)	0.70(.6)
Cisplatin	0.019(.01)	0.0023(.005)	0.0035(.005)

Compound	PC-3	MDA	MCF-7	HT-29
APA	>32	>32	>32	>32
Cp_2TiCl_2	>32	>32	>32	>32
Cp_2Ti/APA	0.71 (.6)	0.71 (.7)	0.74(.7)	0.73(.7)
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/APA	0.64(.6)	0.67(.7)	0.67(.7)	0.69(.7)
Cp_2HfCl_2	>32	>32	>32	>32
Cp_2Hf/APA	0.69(.6)	0.66(.7)	0.70(.7)	0.63(.7)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

The second widely employed measure of the potential use of compounds to inhibit cancer cell growth is the comparison of the ratio of the EC_{50} for the standard cell line WI-38 cells divided by the EC_{50} for the particular test cell. WI-38 cells are normal embryonic human lung fibroblast cells.[93] This value is one of a group called a chemotherapeutic index, CI_{50}. The CI_{50} values for polymers are given in Table 12.

Table 12: CI_{50} values determined from data given in Table 11.

Compound	EC_{50}WI-38/ EC_{50}PANC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}MDA
Cp_2Ti/APA	1.0	1.0	1.0	1.0
Cp_2Zr/APA	0.89	0.89	1.0	0.96
Cp_2Hf/APA	0.93	0.93	0.99	0.99
Cisplatin	8.3	5.4	4.3	6.6

Compound	EC_{50}WI-38/ EC_{50}MDA	EC_{50}WI-38/ EC_{50}MCF-7	EC_{50}WI-38/ EC_{50}HT-29
Cp_2Ti/PDA	1.0	0.96	0.97
Cp_2Zr/PDA	0.96	0.93	0.93
Cp_2Hf/PDA	0.99	0.93	1.0
Cisplatin	6.6	3.3	4.6

When evaluating CI_{50} results, values greater than two are considered significant. From Table 12 there are no values greater than two for the polymers.

In summary, all three polymers exhibit decent inhibition of all of the cancer cell lines based on EC_{50} values but no polymer shows Cl_{50} values greater than two. Based on EC_{50} values the polymers show superior, lower, values compared with both the monomers and cisplatin. There is no agreement as to which values, EC_{50} or Cl_{50}, are preferred when predicting which tested compounds will give better results in live animal studies. Thus, they represent a new group of potentially active anticancer drugs.

COMPARISON

This study is part of ongoing effort to better define easily and readily synthesized agents that can be employed to treat cancers and other undesirable agents such as molds, bacteria, and viruses. We had previously synthesized the analogous organotin analogs of 6-APA.[45] Since one of our major targets is pancreatic acid we tested it against the two human pancreatic cancer cell lines described in the current paper and compared the results. Table 13 contains the results. Most of the organotin polymers showed little or no ability to inhibit the pancreatic cancer cells but the dibutyltin and diphenyltin polymers showed better ability compared to the group 4 metallocene products. In fact, the dibutyltin polymer exhibits ability to inhibit the PANC-1 cells to near the nanogram/mL level and is one of the best found thus far. In fact, from other studies, the organotin products that exhibit the best ability to inhibit the cancer cell lines are the dibutyltin and diphenyltin as found here.

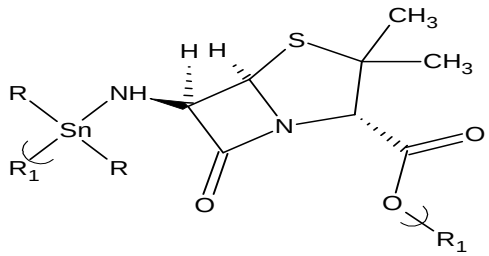


Fig 09: Repeat unit for the product of 6-APA and organotin dihalides.

Table 13: Comparison of the EC_{50} values (micrograms/mL) for the organotin and group 4 metallocene polymers from 6-APA.

Compound	PANC-1	AsPC-1
Me_2Sn/APA	>32	>32
Et_2Sn/APA	>32	0.24(.06)
Bu_2Sn/APA	0.007(.006)	0.16(.06)
Oc_2Sn/APA	>32	>32
Ph_2Sn/APA	0.43(.05)	0.44(.08)
Cp_2Ti/APA	0.72(.6)	0.72(.6)
Cp_2Zr/APA	0.70(.6)	0.70(.6)
Cp_2Hf/APA	0.69(.6)	0.70(.6)

It is advantageous that the dibutyltin polymer shows such good ability to inhibit the pancreatic cancer cell lines for several reasons. Dibutyltin dichloride is widely used in industry as an additive to coatings; it is the least toxic of the organotin monomers to humans; and it the least expensive.

A major reason we carried out this research was to see if the analogous metallocene polymers had better ability to inhibit the pancreatic cancer cell line since in most studies, it was the metallocene polymers that out performed, had better ability to inhibit the cancer cells compared with the analogous organotin polymers. We see here that while the metallocene poly (amine ethers) do exhibit good ability to inhibit the pancreatic cell lines,

that the dibutyltin and diphenyltin poly(amine ether) were the best.

SUMMARY

The poly (amine esters) were produced employing the interfacial polymerization system which is used commercially to produce polycarbonates and aramid fibers.[46,47] The reactants are all commercially available. Thus, there is a straight-forward direction for scale-up.

Infrared analysis shows formation of bands consistent with the formation of the M-N and M-O. MALDI MS shows ion fragment units to seven and eight units in length with isotopic abundant ratios consistent with the presence of metal within the ion fragments. The polymers show good inhibition of all of the human solid cancer cells tested including two pancreatic and two breast human cancer cell lines. Environmentally, group 4 metallocenes are acceptable since they naturally degrade giving the metal oxide.

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