EFFICACY OF A SERIES OF ORGANOMETALLIC POLYMERS AS ANTI-CANCER AND ANTIVIRAL AGENTS

by

LINDSEY CAITLIN MILLER

DISSERTATION

Submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy at

The University of Texas at Arlington

August 2017

Arlington, Texas

Supervising Committee:

Michael Roner, Supervising Professor

Thomas Chrzanowski

Woo-Suk Chang

Paul Chippindale

Shawn Christensen

ABSTRACT

EFFICACY OF A SERIES OF ORGANOMETALLIC
POLYMERS AS ANTI-CANCER AND ANTIVIRAL AGENTS

Lindsey Caitlin Miller, Ph.D.

The University of Texas at Arlington, 2017

Supervising Professor: Michael Roner

Organometallic compounds, particularly organotin compounds, have been known to possess biological activity for over 80 years. A variety of these compounds have demonstrated antimicrobial, anti-cancer, and antiviral activities. With the discovery of the anti-cancer ability of cisplatin, the most widely used chemotherapeutic agent, the interest in the potential capabilities of metal-containing compounds increased substantially. Since then, several metal-containing compounds have been evaluated for their microbiological activity, and our laboratory focuses specifically on organometallic polymers. Our compounds are composed of a metal, especially tin, which acts as the Lewis acid, while the Lewis base in our compounds is provided by a variety of biologically active compounds. Through condensation reactions pioneered by Dr. Charles Carraher (Florida Atlantic University), we have been able to test a variety of organometallic compounds against an array of bacteria, cancer cell lines, and most recently, viruses. The present study summarizes the previous work demonstrating the anti-cancer ability of organometallic compounds, followed by the most recent studies involving the assessment of their antiviral activity. The viruses in this study include vaccinia virus, a double-stranded DNA virus, and Zika virus, a single stranded (+) RNA virus. In terms of anti-cancer ability, the polymers derived from group IV metallocenes show the most promise for future chemotherapeutic use. The polymers with the greatest potential for antiviral use against vaccinia virus are the group III compounds, which are compounds derived from various organotins and 3-amino-1,2-4 triazole. Three compounds have shown promise against Zika virus: compounds KB5, KB8, and FM1. The most promising group of polymers against two

strains of Zika virus (501 and 502) are group II compounds, which are derived from various organotins and camphoric acid.

ACKNOWLEDGEMENTS

I would first like to acknowledge my principal investigator, mentor, and friend, Dr. Michael Roner, without whom none of this research would have been accomplished. Dr. Roner, it has been five years since you took me under your wing. During those five years, I have worked on several research projects. Despite the failures I encountered, your faith in my aptitude as a scientist has never once wavered. Your willingness to help me with the smallest tasks has not gone unnoticed, and I could never thank you enough for all that you have done for me. You have shown me incredible patience and invaluable guidance over years of fighting with my cell cultures. I would also like to thank my current undergraduate assistants, Alison Egert and Caitlynn Reeves. You have been invaluable to me, both in my data collection and in acting as my laboratory emotional support systems when my research would not seem to cooperate. For all the times you came into lab with me on Saturday mornings, thank you.

August 07, 2017

DEDICATION

I dedicate this work to my grandfather, Jidu, and to my parents, Sean and Cheryl. Thank you for supporting me in every endeavor imaginable for 29 years. I would never have accomplished obtaining a doctoral degree without, among other things, your emotional and financial guidance throughout the years. This is for you.

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 14

LIST OF TABLES

Chapter 1

Chapter 14

CHAPTER 1

Organotins and Their Polymers

Introduction

Decades after their introduction, vaccinations remain a contentious topic in society. The recent outbreaks of Zika virus, which lead to an increase in congenital neurological anomalies in the form of microcephaly, emphasize the continued necessity of vaccine research. In recent years, however, the world has seen an increase in immunocompromised individuals who are unable to receive vaccinations.[1] As Drs. Carraher and Roner highlight in their 2008 work *Cisplatin Derivatives as Antiviral Agents,* "Increased longevity and both infectious and environmental factors are producing a population increasingly harboring immunocompromised individuals for whom vaccinations are unworkable".[1] In previous decades, diseases such as HIV and hepatitis C were unavoidably fatal; however, they are now considered chronic conditions that can be effectively managed with immunosuppressive drugs, thus adding patients who are more susceptible to serious infections but for whom most live vaccines are contraindicated. As Ljungman points out in his 2012 paper *Vaccinations of immunocompromised* patients, "The number of patients with immunosuppressed states is increasing. The advances in medical technology result in constant changes in groups of patients undergoing immunosuppressive therapy".[2] Immunologically, live attenuated vaccines (LAVs) usually offer the best protection from potentially fatal diseases, such as measles, mumps, and varicella infections.[3] Live attenuated vaccines, however, do have the theoretical potential to mutate to the wild-type, virulent form of the virus, thereby causing disease, particularly in the immunocompromised population.[3] In a study funded by GlaxoSmithKline and published in 2016 by Varghese *et al,* the necessity of alternative vaccines was highlighted by the estimate that, in 2012, 7.6 million adults in the United States suffered from an immune-debilitating disease (cancer, Crohn's/ulcerative colitis, HIV, renal failure, etc.), and thus, were unable to receive current vaccines.[3] Consequently, it is imperative that new antivirals are created, which, unlike vaccines, possess broad-spectrum activity, allowing both old and new viral diseases to be targeted.[1] In the event of an outbreak with an emerging virus, for example, a virus-specific drug would not be as effective in combating the new

disease as broad-spectrum antivirals, which target a general step in the viral lifecycle.[4] New antiviral therapies will also offer improved drug specificity and a reduction in toxic side effects while simultaneously combating drug resistance.[5] The current research in our laboratory seeks to discover new antiviral drugs in the form of metal-containing polymeric compounds, especially tin-containing compounds (organotins). Most of these compounds have previously shown promising results as anti-cancer agents. Since both cancer and viral infection induce a state of increased cellular replication, I hypothesize that some of these organometallic polymers will also show promise as antiviral agents and could represent a new class of antiviral drugs.

Organotin (IV) compounds were first synthesized by Sir Edward Frankland in 1849 and are still frequently used in industry and agriculture.[6,7] Decades of continued research have demonstrated that organotins possess anti-cancer, antiviral, antibacterial, and anti-inflammatory properties.[7] Chemically, metal-containing compounds contain vacant p, d, and f electron orbitals, which allows for numerous biological interactions.[8] In the 1960s and 1970s, Drs. Morgan and Carraher pioneered the interfacial process, which allows for the rapid and large-scale synthesis of organotin compounds.[8] Throughout the years, our laboratory, under the tutelage of Dr. Michael Roner, has demonstrated a variety of antibacterial, anti-cancer, and antifungal activity for organotin and other metal-containing compounds. My research seeks to further evaluate more of these compounds against vaccinia virus, a double-stranded DNA virus, and Zika virus, a single-stranded, plus-sense RNA virus. Vaccinia virus is the vaccine strain of smallpox and although smallpox has been eradicated worldwide, it remains a threat because of its potential use as a biological weapon. Zika virus remains a serious threat for pregnant women and their unborn fetuses, and no specific antiviral treatment is currently available.

Background

History and Use of Organotins

Organotins, as their name implies, are organic compounds which possess one or more covalent bonds between tin and carbon. The tin-carbon bonds of these compounds are heat and water stable but

can be broken by strong acids or electrophiles.[6] The compounds have the general formula of $R_nSnX_{4-n,}$ where R is an alkyl or aryl group and n is an anionic leaving group, such as chloride or fluoride.[6] The structure of organotins is such that the alkyl group conveys the biological activity of the compound while the X group conveys solubility.[6] Organotin compounds have a variety of uses in agriculture, industry, and as biocidal agents. For example, dibutyltin, dimethyltin, and dioctyltin are extremely heat stable and are utilized in manufacturing PVC pipe to prevent the degradation of the polymeric backbone of PVC.[9] Organotin compounds are also used as fungicides in agriculture (triphenyltin), and as stabilizers in food packaging and coating materials, while tributyltin (TBT) was previously used as an anti-fouling paint additive for ships.[9,10] The toxicity of these compounds is based on the number of organic moieties attached to the tin atom, and thus, trialkyltins are more toxic than dialkytins, which are more toxic than alkyltins.[10] The single and di-substituted alkyltins are used to prevent degradation of PVC piping, while tributyltin (TBT) is used as an anti-fouling paint additive for ships.[10]

Organotin compounds have been known to possess biological activity for over a century, and they are continually tested as novel chemotherapeutic agents.[11] Numerous organotin polymers, including, among others, polyamines, polyesters, and polyethers, have been tested for their anticancer and antimicrobial properties.[8] Furthermore, organotins have been synthesized that combine the polymeric backbone with other biologically-active agents, such as those containing the antiviral drug, acyclovir, and others that contain several antibiotics.[8] It is hypothesized that, by combining the activity of the organotin backbone with a variety of Lewis bases, a synergistic effect will occur.[8] The combination of two biological entities in the polymer increases the likelihood that the microorganism that is being targeted will be effectively destroyed before it can develop resistance to the drug.[12] For example, in a study published in 2006, Carraher *et al.* demonstrated that organotin polymers containing acyclovir, especially dibutyltin polymers, inhibit the replication of herpes simplex virus (HSV) more effectively than acyclovir alone.[12] Previous research in our laboratory has shown that the dibutyltin compounds exhibit the greatest inhibition of cancer cells.[8] Since both cancer and viral infections involve continued DNA replication, it is not unusual for chemotherapeutic agents to also demonstrate antiviral activity.[13] Extensive research has shown a correlation between the efficacy of metal-containing drugs against both cancer cells lines and

viruses. For example, cisplatin, the most frequently used chemotherapeutic agent, has been incorporated into a wide variety of polymers that display both anti-tumor and antiviral properties.[14] Tilorone is a synthetic small molecule that is a potent inducer of the interferon response during early viral infections.[14] In 2008, Roner *et al* demonstrated that cisplatin derivatives of tilorone were effective against both DNA and RNA viruses. The tested viruses included reovirus ST3 (dsRNA), vaccinia virus (dsDNA), HSV-1 (dsDNA), and varicella zoster virus (dsDNA).[14] The tilorone-containing polymers exhibited the best antiviral activity against the DNA viruses. Although vaccinia virus is a DNA virus, it has a unique intracytoplasmic replication cycle, whereas most DNA viruses replicate in the nucleus of the host cell.[14] Only a moderate amount of antiviral activity was seen for vaccinia virus, and thus, it can be surmised that these polymers act on the nuclear RNA polymerase of the host cell.[14] Very little activity of the tilorone derivatives was seen against vaccinia and reovirus, as these viruses both utilize virally-encoded, rather than cellular, RNA polymerases for mRNA production.[14] Reovirus, a double-stranded RNA virus, causes respiratory and enteric infections. Although no antiviral activity was found for single-stranded RNA viruses, the polymers did exhibit activity against reovirus, which requires cellular DNA replication to occur prior to the viral replication cycle.[14] Thus, it appears that the mechanisms which alter cellular processes during cancer are also active during viral infection.[14] The intersection between cancer and virus-related processes is how the present research came into focus.

Organotins as Biological Agents

Organotins have been known to exhibit a variety of biological effects for over 80 years; however, the majority of research in recent decades has been into their use as anti-cancer agents. Organotin compounds in their monomeric form have been tested as potential anti-cancer agents since the 1920s.[15] The most heavily researched tin-containing compound, cisplatin, is a chemotherapeutic agent that was discovered in 1965 and is still utilized to treat a variety of cancers.[5] Although cisplatin is effective against several cancers, the side effects of the drug can be deleterious, and this drug also does not treat some of the most common forms of lung and colon cancers.[5] Cisplatin works through a mechanism of action that prevents cellular DNA replication by inducing crosslinks into the DNA.[5] Viral infections, like cancer, also

require cells to undergo continuous DNA replication, which is why many antiviral and chemotherapeutic agents target the enhanced DNA replication and transcription seen in virus and cancer-infected cells.[5] Since the discovery of cisplatin, numerous other metal-containing compounds have undergone testing for their anti-tumor and antiviral properties. Dr. Charles Carraher, who synthesizes our organotin compounds, began working with them in the 1970s, when he found that a variety of organotin polyethers derived from simple aliphatic diols demonstrated the ability to inhibit cell growth in Balb 3T3 cells.[15] These initial results were the impetus behind the creation and testing of numerous organotins as antimicrobial agents.

Organotin Polymers and Mechanisms of Action

In their monomeric form, tin-containing compounds are highly toxic to a variety of species. For example, although tributyltin (TBT) compounds were previously used as anti-fouling agents in paints, there have since been regulations put in place regarding the use of monomeric tin compounds due to the migration of the tin moiety into the water and the ensuing toxicity to aquatic life.[15] Organotin moieties as part of polymers are much less toxic, and our laboratory focuses on the use of these polymers.[15] The organotin polymers we test consist of a tin or other organometallic moiety coupled with a biologically active Lewis base. The composition of our polymers can be tailored to the specific microorganism being targeted and thus, polymeric drugs offer many advantages over monomeric drugs. The complexity of polymeric drugs, for example, allows for multiple points of interaction with the target cancer cell, microbe, or virus particle.[16] Furthermore, due to their size, polymeric drugs are retained within the body much longer than monomeric drugs, allowing a greater time for the drug to work while simultaneously reducing nephrotoxicity.[8] The complexity of the organotin polymers also allows them to display multiple methods of activity, which increases the likelihood for efficacy and reduces the chance of developing drug resistance.[8] Although the exact molecular targets of organotins have yet to be elucidated, it is known that these compounds are membrane-active, allowing them to cross and embed themselves in biological membranes, and also to work intracellularly on membrane-bound organelles, such as mitochondria.[10] A review of the literature indicates that most biological activity of organotins is due to their effect on membranes and organelles, as well as their ability to disrupt macromolecular synthesis and cellular metabolism. Tri-substituted organotin compounds, for example, appear to prevent mitochondrial

respiration, a process invaluable to the synthesis of the cell's energy source, ATP.[8] Other organotins have been found to prevent DNA and protein synthesis, and also alter the structure of DNA.[7]

Di- and trialkyltin compounds represent the most heavily studied and commercially utilized group of organotin compounds. For example, compounds such as tributyltin chloride and dibutyltin oxide are some of the least toxic organotins towards humans.[15] The structure of organotin compounds allows them to interact with a wide variety of biological molecules, making it difficult to pinpoint the exact targets of their activity.[17] However, it has been found that the most biologically-active organotins (diorganotins with butyl or phenyl moiety) share certain structural features that contribute to their activity, including a combination between hydrophobic and hydrophilic properties, and low hydrolytic cleavage between the carbon and tin atoms of the compounds.[15]

Organotins and other organometallic compounds are non-polar, hydrophobic compounds, which allow them to interact with a variety of biological membranes.[10] Along with disturbing the plasma membrane of cells, organotins have been found to interact with mitochondria, and thus, are known to disrupt ATP synthesis. To review, during ATP synthesis, the oxidation of organic compounds, and thus, the flow of electrons, is coupled to proton extrusion from the matrix of mitochondria, thereby generating an electric potential known as the proton motive force (pmf), which allows for the synthesis of ATP.[17] Compounds, such as organotins, which inhibit the synthesis of ATP may do so through 1) respiratory chain inhibition, 2) ATPase inhibition, or 3) increase in membrane permeability.[17] Detailed studies of each step of ATP production have led to the determination that many organotins, particularly trialkyltin compounds, exert their toxic effects on cells mainly through inhibition of the cellular ATPase necessary for ATP production.[17] It has also been found that intermediates produced by cellular hydrolysis of organotin compounds can bind to cellular macromolecules (DNA, proteins, carbohydrates, lipids).[6] Both triorganotin and methyltin compounds have been found to bind to the cellular ATP-ase system and hemoglobins.[6]

Organometallic Polymers and Their Structures

Table 1 Group I Compounds: Polyamines Derived from Various 4,6-Diaminopyrimidines

Compound Identification	Lewis Acid	Lewis Base
AB1	Bu_2SnCl_2	4,6-diamino-5-isoamyl-2-(3-phenyl propylamino) pyrimidine
AB2	Bu_2SnCl_2	4,6-diamino-5-nitropyrimidine
AB3	Bu_2SnCl_2	4,6-diamino-2-methyl mercaptopyrimidine
AB4	Bu_2SnCl_2	4,6-diamino-2-methyl-5-nitrosopyrimidine
Dibutyltin Dichloride	Organotin monomer	N/A

Table 2 Group II Compounds: Diorganotins + Camphoric Acid

Compound Identification	Lewis Acid	Lewis Base
AC2	Bu_2Sn	Camphoric acid
AC3	Me_2Sn	Camphoric acid
AC4	Et_2Sn	Camphoric acid
AC5	Ph_2Sn	Camphoric acid
AC6	Oc_2Sn	Camphoric acid
Camphoric Acid	N/A	Camphoric acid itself

Table 3 Group III Compounds: Diorganotins + 3-amino-1,2-4 triazole

Compound Identification	Lewis Acid	Lewis Base
RC1	Bu_2Sn	3-amino-1,2-4 triazole
RC2	Me_2Sn	3-amino-1,2-4 triazole
RC3	Et_2Sn	3-amino-1,2-4 triazole
RC4	Ph_2Sn	3-amino-1,2-4 triazole
RC5	Oc_2Sn	3-amino-1,2-4 triazole
3-AT	N/A	3-amino-1,2-4 triazole itself

Table 4 Group IV Compounds: Triphenyl-containing compounds + camphoric acid

Compound Identification	Lewis Acid	Lewis Base
FM1	Ph_3Sb	Camphoric acid
FM2	Ph_3As	Camphoric acid
FM3	Ph_3Bi	Camphoric acid
FM5	Me_2Sn/Lamivudine*	Camphoric acid

*Lamivudine: HIV antiretroviral drug

Table 5 Group V Compounds: Organometallic compounds + dicumarol

Compound Identification	Lewis Acid	Lewis Base
NS1	Ph_2Sn	Dicumarol
NS2	$(C6H2)_2Sn$	Dicumarol
NS3	Et_2Sn	Dicumarol
NS4	Oc_2Sn	Dicumarol
NS5	Cp_2Ti	Dicumarol
NS6	Cp_2Zr	Dicumarol
NS7	Cp_2Hf	Dicumarol
NS8	$Cp2^V$	Dicumarol
JJ4	Bu_2Sn	Dicumarol
JJ5	Me_2Sn	Dicumarol
Dicumarol itself	N/A	Dicumarol itself

Table 6 Group VI Compounds: Organotin dihalides + alpha-cyano-4-hydroxycinnamic acid

Compound Identification	Lewis Acid	Lewis Base
VS1	Bu_2Sn	Cyano-4-hydroxycinnamic acid
VS2	Me_2Sn	Cyano-t-hydroxycinnamic acid
VS3	Et_2Sn	Cyano-4-hydroxycinnamic acid
VS4	Ph_2Sn	Cyano-4-hydroxycinnamic acid
VS5	Oc_2Sn	Cyano-d-hydroxycinnamic acid
CHA	N/A	Cyano-t-hydroxycinnamic acid

Table 7 Group VIIA Compounds: Titanocene + PEGs

Compound Identification	Lewis Acid	Lewis Base
Cp2Ti PEG 200	Cp_2Ti	PEG 200 (S7)
Cp2Ti PEG 400	Cp_2Ti	PEG 400 (2/1S)
Cp2Ti PEG 600	Cp_2Ti	PEG 600 (2/S)
Cp2Ti PEG 1000	Cp_2Ti	PEG 1000 (211)
Cp2Ti PEG 1500	Cp_2Ti	PEG 1500 (Run 4)
Cp2Ti PEG 2000	Cp_2Ti	PEG 2000
Cp2Ti PEG 3400	Cp_2Ti	PEG 3400
Cp2Ti PEG 8000	Cp_2Ti	PEG 8000

Table 8 Group VIIB Compounds: Zirconocene + PEGs

Compound Identification	Lewis Acid	Lewis Base
JF1	Cp_2Zr	PEG 200
JF2	Cp_2Zr	PEG 400
JF3	Cp_2Zr	PEG 1000
JF4	Cp_2Zr	PEG 4600
JF5	Cp_2Zr	PEG 8000

Table 9 Group VIIC Compounds: Hafnocene + PEGs

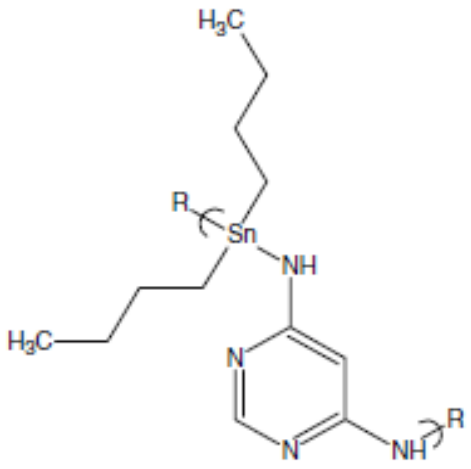
Compound Identification	Lewis Acid	Lewis Base
KB4	Cp_2Hf	PEG 200
KB5	Cp_2Hf	PEG 400
KB6	Cp_2Hf	PEG 1000
KB7	Cp_2Hf	PEG 4600
KB8	Cp_2Hf	PEG 8000

Table 10 Group VIID Compounds: Diphenyl/dibutyltin + PEGs

Compound Identification	Lewis Acid	Lewis Base
ME2	Ph_2Sn	PEG 8000
ME3	Ph_2Sn	PEG 2000 Lowest mol wt
ME4	Ph_2Sn	PEG 2000 Medium 1110 wt
ME5 HIGH	Ph_2Sn	PEG 2000 Highest 11101 wt
ME5 400	Bu_2Sn	PEG 400
ME5 10000	Bu_2Sn	PEG 10000

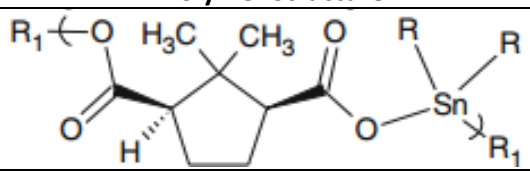
Compound Structures

Figure 1 Group I Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group I: Polyamines derived from various 4,6-diaminopyrimidines	Dibutyltin dichloride	

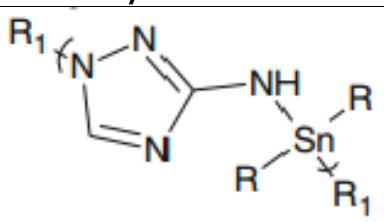
Polymer structure: Carraher et al, 2007[18]

Figure 2 Group II Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group II: Diorganotins plus camphoric acid	Dialkyltins: Butyl, methyl, ethyl, phenyl, octyltin	

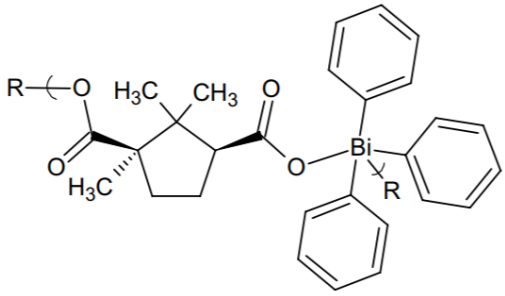
Polymer structure: Carraher and Roner et al, 2015

Figure 3 Group III Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group III: Diorganotins plus 3-amino-1,2-4 triazole	Dialkyltins: Butyl, methyl, ethyl, phenyl, octyltin	

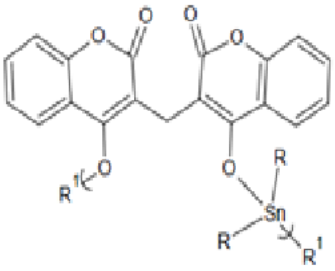
Polymer structure: Carraher and Roner et al, 2015

Figure 4 Group IV Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group IV: Triphenyl-containing compounds plus camphoric acid	Ph_3Sb, Ph_3As, Ph_3Bi	

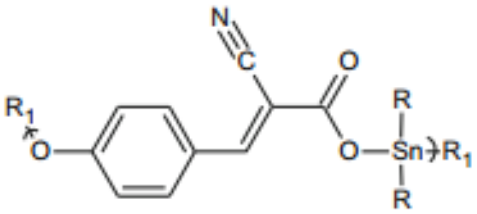
Polymer structure (showing Ph_3Bi): Carraher et al 2017[19]

Figure 5 Group V Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group V: Organometallics plus dicumarol	Variable	

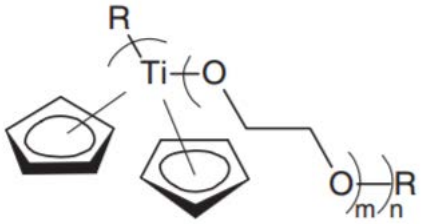
Polymer structure: Carraher and Roner 2009[15]

Figure 6 Group VI Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group VI: Organotin dihalides and Cyano-4-hydroxycinnamic acid	Dialkyltins: Butyl, methyl, ethyl, phenyl, octyltin	

Polymer structure: Carraher and Roner et al 2016[20]

Figure 7 Group VIIA Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group VIIA: Titanocene plus various PEGs	Cp_2Ti	

Polymer structure: Carraher et al, June 2017[19]

Figure 8 Group VIIB Polymer Structure

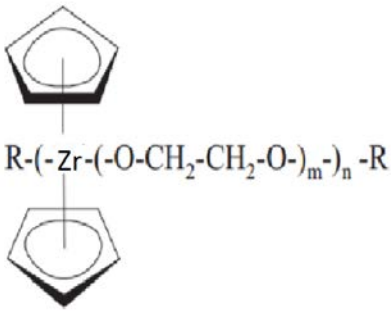
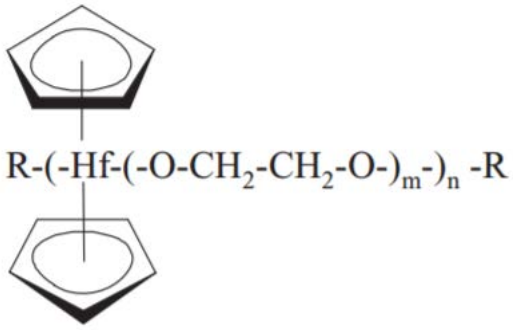
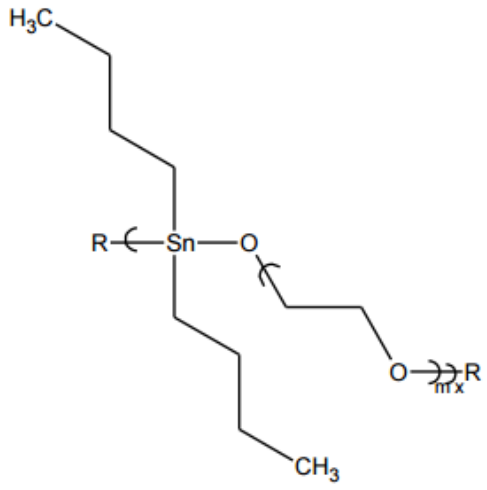
Compound Group	Lewis Acid	Polymer Structure
Group VIIB: Zirconocene plus various PEGs	Cp_2Zr	

Figure 9 Group VIIC Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group VIIC: Hafnocene plus various PEGs	Cp_2Hf	

Polymer structure: Carraher et al, June 2017[19]

Figure 10 Group VIID Polymer Structure

Compound Group	Lewis Acid	Polymer Structure
Group VIID: Diphenyl/dibutyltin plus various PEGs	Diphenyltin, dibutyltin	

Polymer structure (dibutyltin dichloride): Roner and Carraher et al, 2011[13]

References

1) Roner, Michael R., and Charles E. Carraher. "Cisplatin Derivatives as Antiviral Agents." Inorganic and Organometallic Macromolecules (2008): 193-223

2) Ljungman, Per. "Vaccination of Immunocompromised Patients." Clinical Microbiology and Infection 18 (2012): 93-99.

3) Varghese, L., D. Curran, E. Bunge, H. Vroling, F. Van Kessel, A. Guignard, G. Casabona, and A. Olivieri. "Contraindication of Live Vaccines in Immunocompromised Patients: An Estimate of the Number of Affected People in the USA and the UK." Public Health 142 (2017): 46-49.

4) Vigant, Frederic, Nuno C. Santos, and Benhur Lee. "Broad-spectrum Antivirals against Viral Fusion." Nature Reviews Microbiology 13.7 (2015): 426-37.

5) Allardyce, Claire S., and Paul J. Dyson. “Medicinal Properties of Organometallic Compounds.” Topics in Organometallic Chemistry Bioorganometallic Chemistry (2006): 177-210.

6) Nagy, Laszlo, Claudia Pellerito, and Lorenzo Pellerito. “Biological Activity of Organotin(IV) Compounds: Structural and Chemical Aspects.” Biochemical and Biological Effects of Organotins (2012): 25-52.

7) Niu, Lin, Yunlan Li, and Qingshan Li. "Medicinal Properties of Organotin Compounds and Their Limitations Caused by Toxicity." Inorganica Chimica Acta 423 (2014): 2-13.

8) Carraher, Charles E., and Michael R. Roner. "Organotin Polymers as Anticancer and Antiviral Agents." Journal of Organometallic Chemistry751 (2014): 67-82.

9) Giriyan, Asha, and Sangeeta Sonak. "Organotins and Humans: Threat and Risk.” Biochemical and Biological Effects of Organotins (2012): 164-73.

10) White, Jane S., John M. Tobin, and Joseph J. Cooney. "Organotin Compounds and Their Interactions with Microorganisms." Canadian Journal of Microbiology 45.7 (1999): 541-54.

11) Carraher, Charles E., Michael R. Roner, Raven E. Lambert, Luis Arroyo, and Lindsey C. Miller. "Synthesis of Organotin Polyamine Ethers Containing Thiamine (Vitamin B1) and Preliminary Ability to Inhibit Select Cancer Cell Lines." Journal of Inorganic and Organometallic Polymers and Materials J Inorg Organomet Polym 25.6 (2015): 1414-424

12) Carraher, Charles E., Theodore S. Sabir, Michael R. Roner, Kimberly Shahi, Robert E. Bleicher, Joanne L. Roehr, and Kelly D. Bassett. "Synthesis of Organotin Polyamine Ethers Containing Acyclovir and Their Preliminary Anticancer and Antiviral Activity." Journal of Inorganic and Organometallic Polymers and Materials 16.3 (2006): 249-57.

13) Roner, Michael R., Charles E. Carraher Jr., Kimberly Shahi, and Girish Barot. "Antiviral Activity of Metal-Containing Polymers—Organotin and Cisplatin-Like Polymers." Materials 4.12 (2011): 991-1012

14) Roner, Michael R., Charles E. Carraher, Salima Dhanji, and Girish Barot. "Antiviral and Anticancer Activity of Cisplatin Derivatives of Tilorone." Journal of Inorganic and Organometallic Polymers and Materials 18.3 (2008): 374-83.

15) Carraher, Charles E. Jr., and Michael R. Roner. "Organotin Polyethers as Biomaterials." Materials 2.4 (2009): 1558-598.

16) Carraher, Charles E., Michael R. Roner, Kimberly Shahi, Alisa Moric-Johnson, Lindsey Miller, Girish Barot, Amitabh Battin, Nancy T. Trang, and Mohammed H. Al-Huniti. "Control of Prostate Cancer Using Organotin Polymers." Journal of Inorganic and Organometallic Polymers and Materials 25.3 (2014): 386-99.

17) Manente, Sabrina, Alessandra Iero, and Marcantonio Bragadin. “Organotins as Mitochondrial Toxins.” Biochemical and Biological Effects of Organotins (2012): 25-52.

18) Carraher, Charles E., Amitabh Battin, Kimberly R. Shahi, and Michael R. Roner. "Synthesis, Structural Characterization, and Initial Evaluation as Anticancer Drugs of Dibutyltin Polyamines Derived from Various 4,6-Diaminopyrimidines." Journal of Inorganic and Organometallic Polymers and Materials 17.4 (2007): 631-39.

19) Carraher, Charles E., Michael R. Roner, Francesca Mosca, Alisa Moric-Johnson, Lindsey C. Miller, Jeffrey D. Einkauf, Floyd Russell, and Paul Slawek. "Synthesis and Characterization, Including Cancer Cell Line Inhibition, of Group VA (Group 15)-Containing Polyesters from Reaction with Camphoric Acid." Journal of Inorganic and Organometallic Polymers and Materials (2017):

20) Carraher, Charles E., Jr., Michael R. Roner, Ryan Crichton, Alisa Moric-Johnson, Lindsey Miller, Kendra Black, and Floyd Russell. "Synthesis and Preliminary Cancer Cell Line Results for the Product of Organotin Dihalides and Alpha-Cyano-4-Hydroxycinnamic Acid." J Inorg Organomet Polm (2016): 1-11

CHAPTER 2

Organotins as Anti-Cancer Agents: Literature Review

Similarities Between Viral and Cancer-Induced Processes

It is well-established that the steps involved in carcinogenesis rely heavily on dysregulation of the mechanisms that control cellular proliferation. Similarly, most DNA viruses and some RNA viruses rely on increased cellular DNA replication in order access the cellular enzymes necessary for viral replication. The cellular proliferation networks and immune surveillance mechanisms that are present in cancerous cells are also frequently seen in virally-infected cells.[1] In terms of the immune system, the body produces similar inflammatory and T-cell responses to both viruses and cancer.[2] Viruses capable of inducing cancer, known as oncogenic viruses, establish persistent infections through the same proliferation and apoptotic pathways that are seen in non-virally induced cancers.[2] Currently, several viruses are known to induce tumors in humans, such as Epstein-Barr Virus (EBV), numerous papillomaviruses, and Kaposi Sarcoma Herpes Virus (KSHV).[3] Although the tumor viruses represent vastly different virus families, the majority control cellular proliferation through inhibition of the RB1 and p53 tumor-suppressor pathways.[3] Through inhibition of the retinoblastoma (RB1) pathway, viruses push the host cell into S phase of the cell cycle to prepare for viral replication, while inhibiting the p53 pathway prevents cellular apoptosis.[3] These pathways are frequently inactivated in non-infectious cancers, with almost half of all human cancers possessing mutations in the p53 alleles.[4] Given the similarities between viral and cancer infection, it is not surprising that compounds that have previously demonstrated anti-cancer activity are likely to show antiviral activity as well. The connection between anti-cancer and antiviral activities dates back many decades. In 1986, for example, Beres et al demonstrated that a series of 5-alkyl-2'-deoxyuridine 3',5'-cyclic monophosphates (5-R-cdUMPs) possessed both antitumor ability as well as antiviral activity against HSV-1 and HSV-2.[5] It is hypothesized that the organotin compounds used in this research will demonstrate a wide variety of antiviral activity. Further testing of the more active compounds could lead to the creation of novel antiviral drugs.

Simple Organotin Polyethers

A wide variety of organotin polyethers derived from the interfacial polymerization of dibutyltin dichloride and non-cancer inhibitory diols show decent to good inhibition of two human pancreatic cancer cell lines. In view of the general lack of ability of tested compounds to inhibit pancreatic cancer cell lines, this is significant. These cell lines are the AsPC-1 pancreatic cancer cell line which is an adenocarcinoma pancreatic cell line and the PANC-1 pancreatic cancer cell line which is an epithelioid carcinoma pancreatic cell line. The dibutyltin polyethers generally both exhibit EC_{50} values in the same range and lower than cisplatin and higher CI_{50} values than cisplatin. Essentially all of the polymers derived from simple non-aromatic diols showed CI_{50} values greater than 2.

Much of the research into the activity of our organotin polymers has dealt with their potential as novel chemotherapeutic agents, particularly against pancreatic cancer. Pancreatic cancer is often diagnosed at a time when the cancer has already metastasized, and as such, there is no cure for metastatic pancreatic cancer, with most patients only surviving for 3 to 6 months post-diagnosis.[6] Our seminal research into organotin compounds began with the creation of simple organotin polyethers derived from the reaction of non-biologically active diols (ethylene glycol, diethylene glycol, etc.) with dibutyltin.[6] Currently, our polymers are created using organotins and biologically active Lewis bases; however, the original simple polyethers were recently tested for their ability to inhibit pancreatic cancer.[6] Many of the simply dibutyltin polyethers demonstrated effective growth inhibition against two pancreatic cancer cell lines, ASPC-1 and PANC-1.[6] ASPC-1 is derived from a pancreatic adenocarcinoma, while PANC-1 is from an epithelioid carcinoma.[6] Many pancreatic cancers are ductal adenocarcinomas.[6] The organotin polyethers were compared to cisplatin as a control. The chemotherapeutic index for any given compound is the amount of a compound required to inhibit the growth of a normal cell line divided by the amount of a compound needed to inhibit the growth of a cancer cell line. Smaller values indicate that a compound preferentially kills normal cells, while larger values are desired as they indicate a propensity for the tested drug to preferentially kill cancer cells over normal cells.[6] The chemotherapeutic index for

cisplatin against the PANC-1 and ASPC-1 cell lines is 0.04 and 0.01 ng/mL, respectively.[6] Thus, cisplatin is not very effective against these pancreatic cancer cells lines and is also extremely toxic to normal cells. In contrast, chemotherapeutic index values over 2 are considered significant, and many of the organotin polyethers had CI values above 2, with the highest being a CI_{50} of 26 ng/mL for the polymer created from dibutyltin dichloride and 1,7-heptanediol.[6] In prior studies we found that the ability to exhibit decent inhibition of pancreatic cancer was more selective than other cancers we studied including colon, breast, lung, and prostate cancers, yet some of the present polyethers show moderate to good inhibition of the tested pancreatic cancer cell lines. While the diols do not exhibit anticancer activity, the polymers exhibit good anticancer activity based on EC_{50} values. The non-aromatic polymers derived from simple hydroxyl-terminated diols and those derived from ethylene-oxide terminated diols both exhibited good CI_{50} values with most at 2 and greater. The organotin polymers inhibited both cell lines to similar extents consistent with them possibly showing good inhibition to a variety of other pancreatic cancer cells. The results are consistent with the presence of both the dibutyltin moiety and diol-containing moiety critical to achieving good anti-cancer activity.

Dibutyltin Polyamines

Polymers were created using dibutyltin dichloride and derivatives of 4,6-diaminopyrimidine. In choosing a biologically active Lewis base for the creation of our polymers, pyrimidines are essential building blocks for nucleic acids and thus fit our criteria.[7] Furthermore, these polymers were created to evaluate the effects of electron-donating and electron-withdrawing groups on the activity of the polymers.[7] The polymers were tested against a battery of cancer cells including PC-3 cells (prostate cancer), HT-29 (colon cancer), and the breast cancer cells lines MDA MB-231 and MCF-7.[7] Several of these polymers exhibited good inhibition against a variety of the cancer lines. AB1 (4,6-diaminopyrimidine), AB2 (4,6-diamino-5-nitro-pyrimidine), AB3 (4,6-diamino-2-methylmercaptopurine), AB4 (4,6-diamino-2-methyl-5-nitrosopyrimidine, AB5 (4,6-diamino-2-mercapto-pyrimidine), and AB9 (4,6-diamino-5-(4-chloro-phenyl)-6-ethylpyrimidine) showed chemotherapeutic indices against the colon, prostate, and MDA breast cancers well over the preferred value of 2.[7] In comparison, the chemotherapeutic index for cisplatin for these cell lines is much lower. Selected compounds from this class of polymers also showed good inhibition

towards the PANC-1 and ASPC-1 pancreatic cancer cell lines.[8] Given their effectiveness as chemotherapeutic agents, my research tested a handful of these compounds as antiviral agents.

Diorganotins/organometallics plus camphoric acid polymers

Camphoric acid is a derivative of camphor, and it is utilized in medicine to treat nights sweats that commonly present in tuberculosis patients.[9] Through combining organotin dihalides with the disodium salt of camphoric acid, our lab synthesized and test a variety of these polymers against cancer cells lines.[9] For example, these polymers were tested against the PC-3 cell line, which is a human cell line derived from prostate cancer that had metastasized to the bone.[10] Our results showed that the ability of various classes of polymers to inhibit this cancer cell line was heavily dependent on the Lewis base.[10] For the camphoric acid-derived polymers, the lowest EC_{50} was for the diphenyltin-containing polymer, and the highest CI_{50} value was for the ethyltin-containing polymer.[10] Usually, the polymers that exhibit the best activity possess the dibutyltin moiety, but this was not the case for the prostate cancer cell line.[10] Although no specific trend was seen for the polymers as the Lewis base was varied, the range of polymers tested against the PC-3 cell line generally showed better inhibition than the standard chemotherapeutic agent, cisplatin.[10]

Dibutyltins plus 3-amino-1,2,4-triazole

3-Amino-1,2,4-traizole (3-AT) is a biological inhibitor of catalase, which converts hydrogen peroxide into water and oxygen, and it also inhibits one of the enzymes necessary to produce histidine.[11] Using 3-AT as the Lewis base and organotin dihalides as the Lewis acid, our lab has tested several polymers against cancerous cells lines including PC-3, a prostate cancer line.[10] The product derived from octyltin dichloride gave the best results in inhibiting the prostate cancer cell line for this series of polymers. The octyltin and 3 AT-derived polymer showed a low EC_{50} (12,000 ng/mL) for the PC-3 cell line and had a chemotherapeutic index (CI_{50}) over 2 (2.1 ng/mL).[11] Generally, polymers derived from dibutyltin show the best results; however, this was not the case for the PC-3 cell line.[11]

Organometallics plus dicumarol

The efforts described in this article are aimed at designing organotin polymers that control the growth of breast cancer and to identify structure/property relationships that assist in this goal. The growth of MCF-7 and MDA-MB-231 breast cancer cell lines is inhibited employing a wide range of organotin condensation polymers. The EC_{50} values are primarily dependent on the nature of the Lewis base but the CI_{50} is dependent on both the nature of the Lewis base and Lewis acid. A number of products exhibit CI_{50} values greater than two including a number of organotin polyethers such as those derived from diethylstilbestrol, dienestrol, short-chained dibutyltin polyethers, and hydroquinone derivatives. In most of these cases the MDA-MB-231 cells exhibit greater inhibition compared to the estrogen receptor (ER) MCF-7 cells. The organotin polymers generally exhibit a superior ability to inhibit MCF-7 and MDA-MB-231 breast cell growth compared to the standard cisplatin.

Dicumarol is a blood-thinning agent that has been shown to have a variety of biological effects, and thus, satisfies our current aim of using biologically active Lewis bases in our organometallic polymers.[12] Polymers were created by combining organotin dihalides and other organometallic agents with dicumarol, and these polymers were then tested for their anti-proliferative effects against a variety of cancer cell lines. For testing involving breast cancer, MDA-MB-231 (estrogen-receptor negative) and MCF-7 (estrogen-receptor positive) cells were used.[13] For the MDA cell line, the lowest EC_{50} was found for the butyltin and octyltin containing dicumarol polymers.[13] For the MCF cell line, the most promising dicumarol compounds with the lowest EC_{50} results contained butyltin, octyltin, or phenyltin moieties.[13] With much of our effort with organotin-containing polymers, we observed that the most active product contained the dibutyltin moiety. While this was observed for the bank of cancer cells tested by us that included breast, colon, pancreatic, bone, lung, and prostate cancers, is this also true for the MCF-7 and MDA-MB-231 cell lines? Results demonstrated that there is no clear trend with respect to which organotin gives the lowest EC_{50} and highest CI_{50} values within each Lewis base group. Thus, the trend of the best values being found for the dibutyltin-containing polymers is not followed for the MCF-7 and MDA-MB-231 results. In terms of chemotherapeutic index, all the dicumarol-derived polymers showed higher CI_{50} values when compared with cisplatin and can thus be considered significant.

Dialkyltins plus CHA

High polymer poly(ester ethers) are rapidly synthesized in good yield employing the interfacial poly-condensation reaction system. Infrared spectroscopy shows the formation of new bands derived from the Sn-O and Sn-O(CO) linkages. MALDI MS shows formation of ion fragment clusters several units in length. The products show reasonable inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines.

Alpha-cyano-4-hydroxycinnamic acid (CHA) is a natural compound derived from various plant sources.[14] Biologically, CHA prevents the transport of pyruvate across the mitochondrial membrane, thus inhibiting the proper oxidation of glucose. [14] Furthermore, CHA has been shown to possess antimicrobial activity, and it has the potential to control the metabolism of glucose and lipids in the body. [14] Using the interfacial condensation reactions, Dr. Carraher (Florida Atlantic University) synthesized a variety of polymers derived from organotin dihalides and CHA. [14] The organotin moieties used in the polymers included methyltin, ethyltin, butyltin, octyltin, and phenyltin. These polymers were tested against a variety of cancer cell lines for their ability to inhibit cell growth, including pancreatic cancer, breast cancer, prostate cancer, and colon cancer. Cisplatin and CHA itself were also tested against the cell lines for comparison. Results demonstrated that the polymers had effective concentrations (EC_{50}) similar to cisplatin, and are thus considered significant. [14] It is important to note that the polymers showed better inhibition of the two pancreatic cancer cell lines, PANC-1 and AsPC-1, inhibiting the growth of these cell lines at concentrations much lower than those seen for cisplatin. [14]

Water-soluble PEG polymers

Polyethylene glycol (PEG) is a non-toxic substance currently used in pill coatings and laxatives, and it is also highly stable in acidic environments and thus can be administered orally.[15] Due to its inherent water solubility, polyethylene glycol allows for many drugs to be rendered water-soluble, and our PEG-derived organotin polymers were the first water-soluble polymers of their kind.[14] The polymers were created using dibutyltin dichloride and PEG of varying molecular weights, including 400, 8000, and 10,000 Daltons. [10] When testing these compounds against a variety of cancer cell lines and normal cell lines, *Carraher* et al found that the PEG 400 polymer was most toxic to the cells as exhibited by the lower EC_{50} for PEG 400 as

compared to the PEG 8000 and PEG 10,000 polymers.[10] It was hypothesized that the bulkiness of the larger molecular weight polymers may make them more difficult to enter targets cells, and thus, their toxicity is lowered.[10] In terms of their effects on cancerous cells, the polymers generally inhibit cells at values similar to cisplatin; however, as can be seen in the proceeding table, the polymers showed very good inhibition of the pancreatic cancer cell lines.[10]

Significant Findings from Publications

- Simple organotin polyethers created from dibutyltin dichloride and non-cancer inhibitory simple diols showed outstanding inhibition of the two pancreatic cancer cell lines, AsPC-1 and PANC-1.
- Polymers derived from organotin dihalides and alpha-4-hydroxycinnamic acid (CHA) showed better inhibition of the pancreatic cell lines when compared to cisplatin.
- Polymers created from group IVB metallocenes and alpha-4-hydroxycinnamic acid (CHA) showed outstanding inhibition of a variety of cancer cell lines, including pancreatic, breast, and colon cancers. In terms of cancer inhibition, polymers containing hafnocene show the greatest inhibition, followed by zirconocene polymers, and then titanium-containing polymers.
- Polymers created from group IVB metallocenes and 3,5-Pyridinedicarboxylic acid (PA), an inhibitor of bovine liver glutamate dehydrogenase, showed good inhibition of pancreatic, prostate, breast, and colon cancer cell lines. Polymers derived from hafnocene, titanocene, or vanadocene were superior to the polymers containing zirconocene.
- Breast cancer cell lines MDA-MB-231 (estrogen receptor negative) and MCF-7 (estrogen receptor positive) were significantly inhibited by:
    - polymers created from various organotin dihalides and diethylstilbestrol
    - polymers created from dibutyltin dichloride and hydroquinone/hydroquinone derivatives
    - polyethers created from dibutyltin dichloride and 1,1- and 1,2-diols (simple diols)
    - polymers created from various organotin dihalides and histamine
- Prostate cancer cell line PC-3, a cell line derived from prostate cancer that had metastasized to the bone, was significantly inhibited by:
    - polymers created from various organotin dihalides and diethylstilbestrol

- a handful of polymers derived from hydroquinone and hydroquinone derivatives
- polymers from two simple organotin polyethers: dibutyltin dichloride with 2,3-butanediol, and dibutyltin dichloride and 3-chloro-1,2-propanediol

CHAPTER 3

ABILITY OF SIMPLE ORGANOTIN POLYETHERS TO INHIBIT PANCREATIC CANCER[1]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 1-3) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Charles E. Carraher Jr., Michael R. Roner, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot & Nandalall Sookdeo

Charles E. Carraher Jr., Michael R. Roner, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot & Nandalall Sookdeo (**2016**) Ability of simple organotin polyethers to inhibit pancreatic cancer, Journal of Macromolecular Science, Part A, 53:2, 63-71, DOI: 10.1080/10601325.2016.1120172

[1] Used with permission of Springer, 2017

Ability of Simple Organotin Polyethers to Inhibit Pancreatic Cancer

CHARLES E. CARRAHER JR.,[a] MICHAEL R. RONER,[b] ALISA MORIC-JOHNSON,[b] LINDSEY MILLER,[b] GIRISH BAROT,[a] NANDALALL SOOKDEO,[a]

[a]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431

[b]University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Received and Accepted September 2015

A wide variety of organotin polyethers derived from the interfacial polymerization of dibutyltin dichloride and non-cancer inhibitory diols show decent to good inhibition of two human pancreatic cancer cell lines. In view of the general lack of ability of tested compounds to inhibit pancreatic cancer cell lines, this is significant. These cell lines are the AsPC-1 pancreatic cancer cell line which is an adenocarcinoma pancreatic cell line and the PANC-1 pancreatic cancer cell line which is an epithelioid carcinoma pancreatic cell line. The dibutyltin polyethers generally both exhibit EC_{50} values in the same range and lower than cisplatin and higher CI_{50} values than cisplatin. Essentially all of the polymers derived from simple non-aromatic diols showed CI_{50} values greater than 2.

*Address correspondence to: Charles E. Carraher Jr., **Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431:** carraher@fau.edu

Keywords Organotin polymers, tin-containing polymer; pancreatic cancer, AsPC-1 cell line, PANC-1 pancreatic cancer cell line, interfacial polycondensation, anticancer drug

1. Introduction

Cancer is the leading cause of death globally (1, 2). Pancreatic cancer is the fourth leading cause of cancer death in the USA. It afflicts about 32,000 individuals each year in the United States and 168,000 worldwide. Nearly all patients die from the ravages of their disease within 3 to 6 months after detection. Treatment of pancreatic cancer is rarely successful as this disease typically metastasizes prior to detection. Current therapies consist of surgery and, possibly, radiation and chemotherapy. Standard chemotherapy for patients with locally contained cancer includes gemcitabine. Gemcitabine has been shown to improve the quality of life through better pain control, adequate performance status, decreased analgesic consumption, shrinkage of tumor, and prolonged survival. Radiation therapy is usually ineffective except as an adjunct to chemotherapy or as a palliative measure. There is no effective chemotherapy for metastasized pancreatic cancer. 5-Fluorouracil (5-FU) is the most widely used agent for single drug therapy in the treatment of pancreatic cancer. 5-FU is an anti-metabolite that interferes with cellular processes essential for cell division and cell growth. As a result, 5-FU has the greatest effect on rapidly growing cells (3-9). The latest addition to combat pancreatic cancer is the use of

Abraxane, a protein-bound paclitaxel previously used with breast and lung cancer. Coupled with Gemcitabine the combination offers about a two month additional life expectancy to advanced pancreatic cancer patients (10-14).

Two of the most widely used pancreatic cell lines are employed in our studies. These cell lines are AsPC-1 which is an adenocarcinoma pancreatic cell line and PANC-1 which is an epithelioid carcinoma pancreatic cell line (15, 16). Both are human cell lines. About 95% of pancreatic cancers are exocrine pancreatic cancers (ductal adenocarcinoma). The typical cell line used to mimic test compound behavior for this form of cancer is the AsPC-1 pancreatic cancer cell line.

The second pancreatic cancer cell used is the PANC-1 human pancreatic carcinoma, epithelial-like cell line nuclear lysate. It is used as an *in vitro* model of non-endocrine pancreatic cancer for tumorigenicity studies. The cells possess a type B phenotype for glucose-6-phosphate dehydrogenase G6PD. It over-expresses the heregulin/human epidermal growth-factor receptor 2 (HER2/neu) oncogene which is present in 60-70% of human pancreatic carcinomas, but the cell line is estrogen receptor negative.

Work employing organotin small molecules as potential anticancer agents has been ongoing for about 80 years with many contributing (17). These efforts include both the synthesis

and testing of the organotin-containing compounds as to their ability to slow the growth of various cancers and preliminary mechanistic studies identifying various sites where the specific organotin compounds appear to intersect cancer growth. These aspects have been recently reviewed (17-21).

The objective of our studies is to develop compounds that demonstrate good ability to curtail cancer growth, here, pancreatic cancer growth, with minimal harm to the patient. It also involves developing structure/property relationships that will guide further efforts and eventually identify important sites that intersect cancer growth. One major approach taken by us is to employ biologically active reactants, incorporating them within polymers often coupling the biologically active metal-containing moiety with a Lewis base that itself offers biological activity hoping for a synergetic effect by having both biologically active moieties within the same polymer chain. But, we found that a number of simple polyethers derived from non-biologically active diols offered good inhibition of a number of cancer cell lines. At the time of this effort, we had not begun testing cell lines for their ability to inhibit pancreatic cancer. Recently we evaluated these polyethers for their ability to inhibit pancreatic cancer. This paper presents these results.

2 Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the Lewis base and sodium hydroxide 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 room temperature, about 25^{o}C). Stirring was begun and an organic phase (typically 30 mL), generally heptane and the dibutyltin dichloride, rapidly added through a hole in the jar lid using a powder funnel. Addition generally takes less than 3 seconds. 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.

2.2 Physical Characterization

Molecular weight was obtained using Light scattering photometry employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. While not reported here, the structural determination of the polymers was carried out employing the following. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ^{1}H-NMR spectra were obtained in d-6 DMSO employing Varian Inova 400 MHz amd 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 Pro MALDI mass spectrophotometer, Applied Biosystems, Foster City, CA. References describing the synthesis and characterization of the particular polyethers are given in the tables presenting the data.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the PANC-1 human pancreatic duct epithelial carcinoma cell line, AsPC-1 human pancreas ascites adenocarcinoma cell line, and the human normal embryonic lung fibroblast (WI-38) cell line used as the standard. Cells were seeded into a 96-well culture plate at a density of 20,000 cells per well in 100 µL of culture medium. Following a 24 h incubation period, the test compounds were added at concentrations ranging from 0.0032 to 32,000 (or 60,000) ng/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 µL /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 (or in the case of the water soluble organotin polymers in water) and serial dilutions prepared in Minimum 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.

3. Results and Discussion

3.1 Setting

In essentially all studies done by us, polymers containing the dibutyltin moiety are among the most active with respect to inhibiting cancer cell growth. This is significant since it allows the dibutyltin moiety to be used when needed. The dibutyltin moiety is favored for a number of reasons including of the alkytin moieties it is the least toxic towards humans; it has been employed commercially for over 50 years; it is the most commercially available; and it is the least expensive. Further, in nature it degrades to the relatively nontoxic tin oxide (22). In the current study, we employed polyethers containing the dibutyltin moiety.

As noted above, much of our recent effort focused on the coupling of biologically active metal-containing units with biologically active Lewis bases hoping for some synergistic effect. While this is true for most of the recent efforts, it is not true for our efforts with simple diols. This effort was instigated by an observation that even simple diols offered activity against cancer cells. Thus, we synthesized a number of organotin polyethers derived from reaction with various aliphatic and aromatic diols (23-27). We found that a series of organotin polyethers derived from simple aliphatic diols showed good inhibition of Balb 3T3 cells (28, 29). For instance, the product from dibutyltin dichloride and 1,6-hexanediol showed an EC_{50} value of 5000 ng/mL. The product from 1,4-butanediol and dibutyltin dichloride showed an EC_{50}. of 250 ng/mL. And finally, the product of dibutyltin dichloride and 1,4-butenediol showed an EC_{50} value of 25 ng/mL, the lowest GI_{50} found for organotin products at that time. For comparison, the EC_{50} for cisplatin, the most widely used anticancer chemo drug, is 400 ng/mL for this cell line.

This work suggested two structural windows that merited further investigation. These structural windows are that the activity increases as the distance between the oxygen atoms in the diols decreases. Second, that unsaturation, that is the presence of a pi bond in the diol, may contribute to the ability of the organotin polyether to inhibit cell growth. This resulted in the study of a number of organotin polyethers with the results for the pancreatic cancer cell lines included in the present study.

3.2 Standard Biological Measures

Different measures of the ability and effectiveness of drugs to arrest cell growth for cell line studies are employed. The two most widely have been employed in our studies. The first measure of the effectiveness to inhibit cell growth is the effective concentration, EC, to cause growth inhibition for some inhibition fraction, usually the concentration to inhibit the growth by 50%, EC_{50}. The second measure of effectiveness is the chemotherapeutic index, CI, again for some fraction generally 50%, CI_{50}. The chemotherapeutic index is the concentration of the compound that inhibits the growth of the normal cells, usually the NIH/3T3 or WI-38 cells, by 50% divided by the concentration of the compound that inhibits the growth of the cancer cells by 50%. Larger values are desired since they indicate that a larger concentration is required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicate some preference for inhibiting the cancer cells in comparison to the normal cells. There is not general agreement as to which of these two values is the most meaningful when evaluating cancer inhibition.

The EC_{50} and CI_{50} values for the polymer samples are generally compared to the ability of the monomer and some chemo agent to inhibit cell growth. Cisplatin, among the most widely used chemo agents, is normally employed as the standard chemo agent and is employed in the present studies. The values for dibutyltin dichloride and cisplatin are included in Table 1.

3.3 Biological Results

The tables include, along with the biological results, the molecular weight determined from light scattering photometry, and the reference(s) describing synthetic details. None of the diols showed an ability to inhibit the pancreatic cancer cell lines to the concentration limit tested, 32,000 ng/mL. All cell inhibition results are given in ng/mL.

Table 1 contains results looking at the ability to inhibit cell growth for a series of aliphatic diols containing terminal hydroxyl groups separated by methylene units (Table 1 entries 1-7; Fig. 1, left). In general the EC_{50} decreases as the length of the distance between the oxygen atoms increases from 300 ng/mL for ethylene glycol (an average of EC_{50} for the two pancreatic cancer cell lines) to 50 ng/mL for 1,8-octanediol. The most active compound is derived from 1,5-pentanediol with an average EC_{50} of 15 ng/mL. Similar to the EC_{50} trend, the ability to inhibit cancer cell growth based on CI_{50} generally increases as the length between the oxygen atoms increases (that is greater CI_{50} values and better inhibition). We have no explanation for this trend with respect to activity appearing to increase as the distance between the oxygen generally

increases since this corresponds to a lesser concentration of the dibutyltin moiety. It must be noted that while there is this general dependency, the differences are mild.

CI_{50} values of two and greater are considered significant. In almost all cases, CI_{50} values are two and larger for both cell lines. The EC_{50} and CI_{50} values are generally similar for the two cell lines consistent with the compounds intersecting cancer growth in a similar manner. (CI_{50} value of two and greater are made bold in the tables for easy identification.) This may indicate that these compounds may show good inhibition of other pancreatic cancer cell lines.

Table 1. Cell growth for dibutyltin polyethers (30-35)

PN*	**Lewis Acid**	**Lewis Base**	**Mol. Weight**	**EC_{50} WI-38**	**EC_{50} PANC-1**	**CI_{50} PANC-1**	**EC_{50} AsPC-1**	**CI_{50} AsPC-1**
1	Bu_2SnCl_2	Ethylene Glycol	1.0×10^4	1700(200)	270(20)	**6.3**	330(20)	**5.2**
2	Bu_2SnCl_2	1,3-Propanediol	3.0×10^4	1000(100)	170(20)	**5.9**	210(10)	**4.8**
3	Bu_2SnCl_2	1,4-Butanediol		110(9)	43(10)	**2.6**	27(20)	**4.1**
4	Bu_2SnCl_2	1,5-Pentandeiol	1.8×10^4	200(90)	12(1)	**17**	19(1)	**11**
5	Bu_2SnCl_2	1,6-Hexanediol	2.1×10^5	3500(1000)	1100(700)	**3.2**	890(220)	**3.9**
6	Bu_2SnCl_2	1,7-Heptanediol	1.2×10^4	950(100)	37(20)	**26**	550(100)	1.7
7	Bu_2SnCl_2	1,8-Octanediol	4.0×10^4	480(200)	23(10)	**21**	77(20)	**6.2**
8	Bu_2SnCl_2	Diethylene Glycol	1.1×10^5	1500(100)	330(10)	**4.5**	260(60)	**5.8**

9	Bu_2SnCl_2	Triethylene Glycol	1.7×10^5	1500(100)	300(30)	**5**	220(80)	**6.8**
10	Bu_2SnCl_2	Pentaethylene Glycol	4.7×10^5	1100(100)	940(60)	1.2	1100(600)	1.0
11	Bu_2SnCl_2	PEG-400	7.6×10^4	1340(110)	220(20)	**6.1**	130(30)	**10**
12	Bu_2SnCl_2	PEG-8,000	1.1×10^5	1800(140)	460(40)	**3.9**	330(20)	**5.5**
13	Bu_2SnCl_2	PEH-10,000	7.6×10^4	1000(200)	50(10)	**20**	60(10)	**20**
14	Bu_2SnCl_2	2,5-Dimethyl-6-hexyne-2,5-diol	1.35×10^5	20(1)	2100(200)	0.01	1700(200)	0.01
15	Bu_2SnCl_2	2-Butyne-1,4-diol	1.2×10^5	40(1)	2000(200)	0.02	1400(200)	0.03
	Cisplatin			15(10)	340(12)	0.04	1400(150)	0.01
	Bu_2SnCl_2			200(50)	3.2(1)	**62**	12(1)	**17**

PN* = Product Number

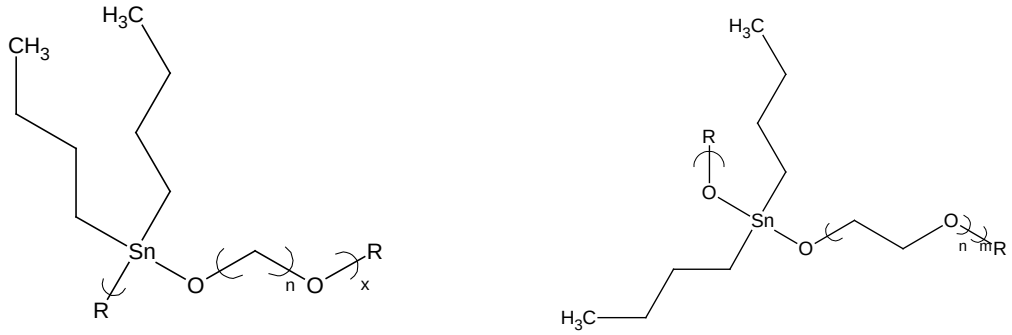


Fig. 1. Repeat unit for the polyethers derived from reaction of dibutyltin dichloride and various hydroxyl-terminated ethylene oxides (right) and methylene spacer diols (left) where R represents chain extension.

Table 1 also contains results as the number of ethylene oxide units are increased (Table 1 entries 1, 8-13; Fig. 1 right). Entries 11-13 are derived from reaction of dibutyltin dichloride with hydroxyl-capped polyethylene glycol (also called polyethylene oxide). These products represent the first reported water soluble organotin polymers and are significant since they allow ready use as a medication through any of the traditional routes. In general, for most of the cancer cell lines studied, these water-soluble polymers exhibit good activity, but for the prostate cell line they offer only moderate ability to inhibit cancer growth. For the PEG water soluble products the PEG 1000 product shows the lowest EC_{50} and highest CI_{50} yet the distance between the dibutyltin units is greatest. This is counter to what one might think but similar to that observed when varying the methylene units. As the distance between the connective oxygen increases the organotin content decreases so if the organotin moiety were the main deciding factor, then it would be expected that the ability to inhibit cell growth would decrease, not increase. Thus, it is the combination of the organotin moiety with the diol-derived moiety that is critical to the anticancer activity.

The last two entries in Table 1 are for two diols containing unsaturation, triple bonds. These two compounds exhibit good inhibition of non-pancreatic cancer cell lines but in both cases with the pancreatic cancer cells there are reasonably high EC_{50} and low CI_{50} values showing low anticancer ability. This is consistent with the ability to inhibit pancreatic cancer cells being more difficult compared to other cancer cell lines. And it is not consistent with the notion that the presence of unsaturation results in increased ability to inhibit pancreatic cancer cell growth.

Another way to introduce unsaturation into the polymer chain is to employ Lewis bases that are aromatic. Hydroquinone and substituted hydroquinones fulfill this requirement (Fig. 2). Table 2 contains results of such a study. The hydroquinones are arranged in increasing electronegativity. There appears to be little correlation between electronegativity and ability to inhibit pancreatic cancer cell growth. For prostate PC-3 cancer cells we found that there appeared to be a relationship between the steric nature of the hydroquinone and PC-3 growth such that the ability to inhibit PC-3 growth as measured by both EC_{50} and CI_{50} is decreased as the bulk on the hydroquinone increases (21). Thus, the tert-butylhydroquinone and 2,5-di-tert-butylhydroquinone polymers show relatively high EC_{50} and low CI_{50} values while the less bulky methylhydroquinone exhibits relatively low EC_{50} and high CI_{50} values. The influence of bulk is also found in comparing the halogen-containing hydroquinone derivatives. Thus, the more bulky bromohydroquinone, 2,5-dichlorohydroquinone and tetrachlorohydroquinone products show higher EC_{50} and lower CI_{50} values compared with the chlorohydroquinone polymer. The relationship between bulk and ability to inhibit PC-3 growth is consistent with size being important in the inhibition of the PC-3 cells. This is consistent with the presence of a size constraint for some important step in cancer growth. (The various mechanisms reported for cancer inhibition by organotin materials have been recently reviewed (17)). Additionally, this steric dependence does not exist with other cancer cell lines tested so is characteristic of the prostate PC-3 cancer cell line. This relationship between bulk and ability to inhibit pancreatic cell growth is not apparent for the pancreatic cancer cell lines.

Table 2. Results for the dibutyltin polyethers derived from hydroquinone and the hydroquinone derivatives (36)

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PANC-1**	**CI_{50} PANC-1**	**EC_{50} AsPC-1**	**CI_{50} AsPC-1**
Bu_2SnCl_2	Methoxyhydroquinone	4.2×10^4	280(30)	1000(100)	0.3	770(100)	0.4
Bu_2SnCl_2	Tert-Butylhydroquinone	3.3×10^4	280(30)	3200(300)	0.09	2900(300)	0.1
Bu_2SnCl_2	2,5-Di-tert-Butylhydroquinone	2.0×10^4	390(30)	3500(300)	0.1	2000(0200)	0.2
Bu_2SnCl_2	Methylhydroquinone	2.9×10^4	220(20)	610(100)	0.4	520(100)	0.4
Bu_2SnCl_2	Phenylhydroquinone	3.3×10^4	60(1)	1100(100)	0.05	890(0100)	0.07
Bu_2SnCl_2	Hydroquinone	2.2×10^4	180(10)	830(100)	0.2	770(100)	0.2
Bu_2SnCl_2	2,3-Dicyanohydroquinone	8.3×10^4	160(10)	1300(100)	0.1	1400(100)	0.1
Bu_2SnCl_2	Bromphydroquinone	2.6×10^4	150(10)	2000(200)	0.1	1800(200)	0.1
Bu_2SnCl_2	Chlorohydroquinone	5.9×10^4	110(10)	580(100)	0.2	480(50)	0.2
Bu_2SnCl_2	2,5-Dichlorohydroquinone	2.5×10^4	380(40)	410(100)	0.9	310(40)	1.2
Bu_2SnCl_2	Tetrachlorohydroquinone	1.9×10^5	440(40)	320(100)	1.4	110(10)	**4.0**
Bu_2SnCl_2	2,5-Dihydroxybenzaldehyde	6.8×10^4	240(20)	1200(100)	0.2	1100(100)	0.2

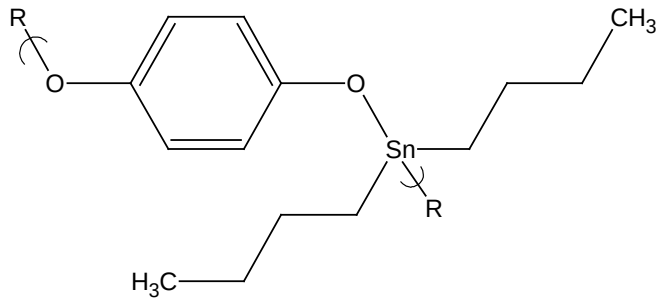


Fig. 2. Repeat unit for the product of organotin dihalides and hydroquinone where R represents chain extension

The initial premise studied indicated that value is gained when unsaturation is present in the diol. This is not borne out for the ability of the hydroquinone derivatives to inhibit the pancreatic cancer cell lines based on CI_{50} values but they do exhibit decent EC_{50} values. While the hydroquinone-containing polyethers exhibit inhibition of the pancreatic cells based on EC_{50} values, the extent of inhibition is inferior to that observed for the simple diols described in Table 1. Further, while these hydroquinone and hydroquinone derivatives exhibit good inhibition of other cancer cell lines, they do not show good ability to inhibit pancreatic cancer cell growth.

Table 3 contains values for diols where the distance between the oxygen atoms is small. For all three polymers the EC_{50} values are somewhat low but none of them exhibit high CI_{50} values. The premise that shortening the distance between the oxygen atoms should promote greater inhibition is not borne out with respect to these products and pancreatic cancer.

Table 3. Product biological activities for organotin polyethers derived from 1,2-diols

(36, 37)

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PANC-1	CI_{50} PANC-1	EC_{50} AsPC-1	CI_{50} AsPC-1
Bu_2SnCl_2	2,3-Butanediol	5.6×10^4	30(1)	2100(200)	0.01	1800(200)	0.01
Bu_2SnCl_2	3-Chloro-1,2-propanediol	4.5×10^4	30(1)	1900(200)	0.02	1400(200)	0.02
Bu_2SnCl_2	Neopentyle Glycol	8.1×10^4	20(1)	2100(200)	0.01	1200(100)	0.02

4. Conclusions

A variety of organotin polyethers were evaluated with respect to their ability to inhibit the two most widely used human cancer cell lines- AsPC-1 pancreatic cancer cell line which is an adenocarcinoma pancreatic cell line and the PANC-1 pancreatic cancer cell line which is an epithelioid carcinoma pancreatic cell line. In prior studies we found that the ability to exhibit decent inhibition of pancreatic cancer was more selective than other cancers we studied including colon, breast, lung, and prostate cancers, yet some of the present polyethers show moderate to good inhibition of the tested pancreatic cancer cell lines. While the diols do not exhibit anticancer activity, the polymers exhibit good anticancer activity based on EC_{50} values. The non-aromatic polymers derived from simple hydroxyl-terminated diols and those derived from ethylene-oxide terminated diols both exhibited good CI_{50} values with most at 2 and greater. The organotin polymers inhibited both cell lines to similar extents consistent with them possibly

showing good inhibition to a variety of other pancreatic cancer cells. Those derived from hydroquinone and hydroquinone derivatives exhibit decent EC_{50} values but only one exhibited a CI_{50} greater than 2. Also, dibutyltin polyethers derived from short-chained 1,2-diols showed decent inhibition of the cell lines based on EC_{50} values but none exhibited CI_{50} values greater than 2. The results are consistent with the presence of both the dibutyltin moiety and diol-containing moiety critical to achieving good anticancer activity.

The simple non-aromatic diols are currently being evaluated for further studies. In particular, the water soluble polyethers have been selected for animal studies since their water solubility allows for simply employing water solutions for administration of the drug.

References

1. Siegel, R., Ward, E., Brawley, O., Jemal, A. (2011) J. Clin., 61, 212.
2. Hariharan, D., Saied, A., Kocher, H. M. (2008) HPB 10**,** 58.
3. Wanebo, H. J., Vexeridis, M. P. (1996) Cancer, 78, 580,
4. Warshaw, A. L., Fernandez-DelCastillo, C. (1992) N. Eng. J. Med., 326, 580.
5. Moertel, C. G. (1976) Clin. Gastroenterol., 5, 777.
6. DeCaprio, J. A., Mayer, R. J., (1991) J. Clin. Oncol., 9, 212.
7. Burris, H. A., Moore, J., Andersen, J., Green, M., Rothenberg, M., Modiano, M., Cripps, M., Portenoy, R., Storniolo, A., Tarassoff, P. (1997) J. Clin. Oncology, 15, 2403.
8. Rothenberg, M. L., Sharma, A., Burris, H. A. (1998) Annals Oncology, 9, 733.

9. Hui, Y. F., Reitz, J. (1997) Am. J. Health-Syst. Pharm., 54, 162.

10. Gradishar, W. (2006) J. Expert Opin. Pharmacother., 7(8), 1041.

11. Green, M. R. (2006) Ann. Oncol., 17(8), 1263.

12. Miele, E. (2009) Int. J. Nanomedicine, 4, 99.

13. Stinchcombe, T. E. (2007) Cancer Chemother. Pharmacol., 60(5), 759.

14. Von Hoff, D. D., (2013) N. Engl. J. Med., 369(18), 1691.

15. Deer, E., Gonzalez-Hermandez, J., Coursen, J., Shea, J., Ngatia, J., Scaife, C., Firpo, M., Mulvihill, S. (2010) Pancreas, 39(4), 425-435.

16. Ulrichy, A. B., Schmied, B. M., Standop, J., Schneider, M. B., Pour, P. M. (2002) Pancreas, 24(2), 111-120.

17. Carraher, C., Roner, M. (2014) J. Organomet. Chem., 751, 67.

18. Carraher, C. Macromolecules Containing Metal and Metal-Like Elements. Vol. 4. Group IVA Polymers, Wiley: Hoboken, NJ, (2005), pp. 263-310.

19. Carraher, C., Roner, M. Shahi, S., Barot, G. (2011) Materials, 4, 801.

20. Carraher, C., Roner, M. (2009) Materials, 2, 1558.

21. Carraher, C., Roner, M. K., Shahi, S., Moric-Johnson, A., Miller, L., Barot, G., Battin, A., Trang, N., Alhumiti, M. (2015) J. Inorg. Organometal. Polym., 25, 386.

22. Hoch, M. (2001) Appl. Geochem., 16, 719.

23. Shahi, K., Roner, M., Barot, G., Carraher, C. (2008) Poly. Mater. Sci. Eng., 98, 390.

24. Carraher, C., Lee, J. L. (2004) Polym. Mater. Sci. Eng., 90, 408.

25. Carraher, C., Scherubel, G. (1972) Makromolekulare Chemie, 152, 61.

26. Carraher, C., Scherubel, G. (1972) Makromolekulare Chemie, 160, 259.

27. Carraher, C., Scherubel, G. (1971) J. Polymer Sci., 9(A1), 9858.
28. Siegmann-Louda, D., Carraher, C., Nagy, D., Snedden, D., Rosa, (2003) J. Polym. Mater. Sci. Eng., 89, 487.
29. Barot, G., Siegmann-Louda, D., Zevalias, E., Carraher, C. (2006) Polym. Mater. Sci. Eng., 94, 561 .
30. Barot, G., Shahi, K., Roner, M., Carraher, C. (2007) J. Inorg. Organometal. Polym., 17, 595.
31. Carraher, C., Barot, G., Shahi, K., Roner, M. (2013) J. Chinese Adv. Maters., 1, 294.
32. Carraher, C., Roner, M., Shahi, K., Battin, A., Barot, G., Arnold, T. (2014) J. Polym. Mater., 31, 1.
33. Carraher, C., Barot, G., Roner, M., Shahi, K., Pellerito, C., Fiore, T., Scopelito, M., Pellerito, L. (2012) J. Polym. Mater., 29, 361.
34. Carraher, C., Barot, G., Shahi, K., Roner, M. (2006) J. Polymer. Mat., 23, 423.
35. Roner, M., Shahi, K., Barot, G., Batton, A., Carraher, C. (2009) J. Inorg. Organomet. Polym., 19**,** 410.
36. Barot, G., Roner, M., Naoshima, Y., Nagao, K., Shahi, K., Carraher, C. (2009) J. Inorg. Organometal. Polym., 19, 12.
37. Shahi, K., Roner, M., Barot, G., Carraher, C. (2008) Poly. Mater. Sci. Eng., 99**,** 349.

CHAPTER 4

CONTROL OF PROSTATE CANCER USING ORGANOTIN POLYMERS[2]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 1-19) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher, Charles E., Michael R. Roner, Kimberly Shahi, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot, Amitabh Battin, Nancy T. Trang, and Mohammed H. Al-Huniti

Carraher, Charles E., Michael R. Roner, Kimberly Shahi, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot, Amitabh Battin, Nancy T. Trang, and Mohammed H. Al-Huniti **(2015)** "Control of Prostate Cancer Using Organotin Polymers." Journal of Inorganic and Organometallic Polymers and Materials, 25, 386-399.

‘

[2] Used with permission of Taylor & Francis, 2017

Control of Prostate Cancer Using Organotin Polymers

Charles E. Carraher Jr., [a] Michael R. Roner,[b] Kimberly Shahi,[b] Alisa Moric-Johnson,[b] Lindsey Miller, Girish Barot, Amitabh Battin,[a] Nancy T. Trang,[b] Mohammed H. Al-Huniti[a]

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 and Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410 (carraher@fau.edu**)**

b. University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Abstract The growth of prostate cancer is affected employing a wide range of organotin condensation polymers. The EC_{50} values are primarily dependant on the nature of the Lewis base but the CI_{50} is dependent on both the nature of the Lewis base and Lewis acid. EC_{50} values down to the picogram/mL range are found. A number of products exhibit CI_{50} values greater than two. For polymers derived from hydroquinone and hydroquinone derivatives ability to inhibit cancer cell growth decreases as the bulk of substituents increases.

Keywords Organotin polymers; tin-containing polymer; prostate cancer; cancer interfacial polycondensation, anticancer drugs

Running title Control Prostate Cancer with Organotin Polymers

Contribution to the memory of Eberhard Neuse special issue.

1Introduction

1.1 General

Cancer is the leading cause of death globally.[1,2] While there have been great strides made in the detection and treatment of prostate cancer, globally it is the sixth leading cause of cancer-related death in men. In the USA it is the second leading cause of cancer-related death.[1,2] It was the cause of the death of Eberhard Neuse and this paper is offered as a remembrance honoring his life.

Detection of prostate cancer is usually indicated by symptoms, physical examination, prostate-specific antigen (PSA), and biopsy. The management of prostate cancer is guided by severity. Low risk tumors are often followed with surveillance without further treatment until called for. Curative treatment is quite varied and includes surgery, radiation, proton therapy, cryosurgery, hormonal therapy, and use of chemotherapy. Our current focus is on developing drugs for chemotherapy.

There are no "magic bullets" for the treatment of advanced metastatic prostate cancer. The chemo-agents employed to treat advanced stage prostate cancer offer side effects similar to those found for other cancers since the chemodrugs are generally the same as used for other cancers. Those approved for prostate cancer include Abiraterone acetate, Bicalutamide, Cabazitaxel, Casodex, Degarelix, Denosumab, Docetaxel, Enzalutamide, Jevtana, Leuproilide acetate, Lupron, Lupron Depot, Prednioson, Prolia, Provenge, Sipuleucel-T , Taxotere, Viadur, Xgeva, Xofigo, Xtandi, and Zytiga.[3] The most common side effects include loss of appetite, hair loss, nausea, vomiting, diarrhea, fatigue, infertility, and mouth sores.[4] Thus, there is a great need for drugs that effectively treat advanced stage prostate cancer with less negative side-effects.

There are a number of cell lines employed in prostate cancer research.[4] The most widely used is the PC-3 (ATCC CLR-1435) cell line which is the cell line employed by us and reported on in the current paper. It is a human prostate cell line derived from metastatic prostate cancer in the bone.[5,6] It is characteristic of prostatic small cell carcinoma, the most common form of advanced prostate cancer.[5,6]

We have synthesized many metal-containing polymers for control of various microbes including bacteria, fungus, virus, and cancer. Much of this has been recently reviewed.[7-12] Here we will focus on tin-containing polymers.

One major approach taken by us is to employ biologically active reactants, incorporating them within polymers often coupling the biologically active metal-containing moiety with a Lewis base that itself offers biological activity hoping for a synergetic effect by having both biologically active moieties within the same polymer chain.

As part of the biological characterization of many of the recently synthesized organotin polymers we included the ability to inhibit a battery of human-cancer cell lines including the PC-3 prostate cell line. The current paper examines the results of these studies focusing on identifying the possible influence of certain structural features to inhibit PC-3 cancer cell growth.

1.2 Advantages of Polymer Drugs

The advantages of polymeric drugs have been recently reviewed emphasizing their use in combating cancer.[8-10,13-18] Following briefly describes some of these advantages in comparison to small, monomeric drugs.

First, polymers should be filtered out by the kidneys more slowly than small compounds[19] decreasing kidney damage and increasing body retention time. This should allow a greater time for the cancer to be attacked by the polymeric drug. Second, a large polymer may be effective against tumors that have developed resistance to other chemotherapeutic agents, either because the polymer is not recognized by cellular resistance mechanisms or because the polymer could be used to deliver local, high-dose chemotherapy.[20] This is particularly important in the treatment of so called resistant cancers that have developed resistance subsequent to initial treatment of by chemo agents. This developed resistance is normal in chemo patients. Our polymers have shown good inhibition of such resistant cancer cell lines.[21] Third, the size and structure of the polymer should provide more binding sites to cellular targets, increasing effectiveness. Fourth, polymers can be designed to incorporate multiple anticancer agents within the same molecule that act against cancer cells by different mechanisms. Our practice of incorporating biologically active Lewis bases along with the organotin moiety has provided a number of polymers with good ability to inhibit cancer cell growth. Fifth, the polymeric structure permits easy coupling to other molecules, such as those that specifically target cancer cells, allowing delivery of a polymeric drug to a particular site without impairing its effectiveness. Sixth, polymers can be designed as either large stable compounds that enter the cell by pinocytosis [22,23] and is active in a polymeric form, or as an unstable compound that degrades slowly into active monomeric units in some timed-release fashion. [24-26] Seventh, large molecules accumulate in solid tumors more than in normal tissues because of the enhanced permeability and retention effect. This results in high amounts of the polymer occupying the interstitial space due to the leaky vasculature and limited lymphatic drainage typical of tumors.[26] This effect is referred to as the enhanced permeability and retention effect, EPR effect. [16-18,26] Finally, polymers can be synthesized to fine-tune structural characteristics, such as monomeric

components, chain length, cross-linking, and polarity, to maximize anticancer activity and vary the spectrum of activity. Thus, polymers offer a wide degree of flexibility in their design and many possible benefits compared to monomeric drugs.

2 Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the Lewis base and sodium hydroxide 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 room temperature, about 25 ^{o}C). Stirring was begun and an organic phase (typically 30 mL), generally heptane and the organotin dihalide, rapidly added through a hole in the jar lid using a powder funnel. Addition generally takes less than 3 seconds. 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.

2.2 Physical Characterization

Molecular weight was obtained using Light scattering photometry employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. While not reported here, the structural determination of the polymers was carried out employing the following. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ^{1}H NMR spectra were obtained in d-6 DMSO employing Varian Inova 400 MHz amd 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 Pro MALDI mass spectrophotometer, Applied Biosystems, Foster City, CA.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human prostate carcinoma (PC-3) and human normal embryonic lung fibroblast (WI-38) cell line used as the standard. Cells were seeded into a 96-well culture plate at a density of 20,000 cells per well in 100 µL of culture medium. Following a 24 h incubation period, the test compounds were added at concentrations ranging from 0.0032 to 32,000 (or 60,000) ng/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 µL /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 (or in the case of the water soluble organotin polymers in water) and serial dilutions prepared in MEM to treat the cells, then the mock-treated cells were "treated" with the same serial dilutions of DMSO without added chemotherapeutic compound. This was done to ensure that any cytotoxicity observed was due to the activity of the compound and not the diluents. For the studies reported here, the mock-treatment never resulted in a loss of cell viability of more than 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.

3 Results and Discussion

3.1 Standard Biological Measures

Different measures of the ability and effectiveness of drugs to arrest cell growth for cell line studies are employed. The two most widely have been employed in our studies. The first measure of the effectiveness to inhibit cell growth is the effective concentration, EC, to cause growth inhibition for some inhibition fraction, usually the concentration to inhibit the growth by 50%, EC_{50}. The second measure of effectiveness is the chemotherapeutic index, CI, again for some fraction generally 50%, CI_{50}. The chemotherapeutic index is the concentration of the compound that inhibits the growth of the normal cells, usually the NIH/3T3 or WI-38 cells, by 50% divided by the concentration of the compound that inhibits the growth of the cancer cells by 50%. Larger values are desired since they indicate that a larger concentration is required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicate some preference for inhibiting the cancer cells in comparison to the normal cells. There is not general agreement as to which of these two values is the most meaningful when evaluating cancer inhibition.

The EC_{50} and CI_{50} values for the polymer samples are generally compared to the ability of the monomer and some chemo agent to inhibit cell growth. Cisplatin, among the most widely used chemo agents, is normally employed as the standard chemo agent and is employed in the present studies.

3.2 Inhibition Results

Tables 1-12 and 15-19 contain PC-3 results for polymers grouped together according to the particular Lewis base. Each set also has references included that give the original synthesis for these materials. The EC_{50} values are given in nanograms/mL. CI_{50} values of two and greater are considered significant and given in bold. The Lewis bases typically offer little or no inhibition of the PC-3 cell line. Where this is not the case, the inhibition values are included. The repeat unit for each group is given following the appropriate table. Inhibition by the organotin monomers is given in Table 13. Tables 1-13 and 15-19 contain the EC_{50} for the standard, here WI-38 cells, and for the particular compound tested. They also contain the CI_{50} which is the EC_{50} WI-38/EC_{50} PC-3.

Table 13 contains the best (lowest) EC_{50} values and (highest) CI_{50} values for each of the different Lewis base groups. Several observations are apparent. First, the inhibition indicators, EC_{50} and CI_{50} values, vary according to the nature of both the organotin and Lewis base. Second, with much of our effort with organotin-containing polymers, we observed that the most active products contained the dibutyltin moiety. While this was observed for the bank of cancer cells tested by us that included breast, colon, pancreatic, bone, lung, and prostrate cancers, is this also true for the PC-3 cell line? We see that there is no clear trend with respect to which organotin gives the lowest EC and highest CI values within each Lewis base grouping. Thus, the trend of the best values being found for the dibutyltin-containing polymers is not followed for the PC-3 results. In comparison to the standard cisplatin, the EC and CI values are generally superior. Third, the employed Lewis bases offer a broad range of connective groups to the organotin moiety including Sn-O, Sn-N and Sn-O-C(O) as well as mixed linkages. Thus, they represent all of the most

common linkages for linear organotin polycondensation polymers. There does not appear to be any linkage that is markedly superior or inferior to inhibiting the PC-3 prostate cancer cell line.

In looking at the data from Tables 1-12 there is a wide range of EC_{50} values from >32,000 mg/mL, the highest concentration tested for several polymers, to 0.0036 mg/mL for the dimethyltin/isomannide product. This last value is within the picogram/mL range (3.6 picogram/mL) about 10^6 less than the EC_{50} value for cisplatin . Within a given group designated by a particular Lewis base, the EC_{50} values are generally similar. Thus, to a first approximation, the ability to inhibit PC-3 cancer cells is mostly dependent on the nature of the Lewis base. As noted before, in most cases, the EC_{50} values are lower than for cisplatin. In general, CI_{50} values of 2 and greater are considered significant. Looking at only the highest value results given in Table 13, there are only 4 out of the possible 12 Lewis base groups that have values of 2 and greater. The CI_{50} for cisplatin is only 0.05. It is interesting that there is not a general agreement between the compounds within a Lewis base grouping with respect to which polymer gives the lowest EC_{50} and the highest Cl_{50} value. This is consistent with the general difference within the cancer study groups as to which measure is more meaningful.

The tables also contain the average molecular weights for each of the polymers. We have found that the ability to inhibit various cancer cell lines is not primarily dependent on chain length (Table 15).[41] We have also found that the active agent is the intact polymer chain .[42]

For the products given in Table 15, as chain length changes presumably changes of not only chain size occurs, but also the particular fraction of end group with a particular end group also changes. Since the EC_{50} and CI_{50} values are similar, ability to inhibit cancer growth appears to be largely

independent of both chain length and nature of the end group for a given Lewis acid/base pair. More study is needed before this is more confidently accepted as true.

As noted above, much of our recent effort focused on the coupling of biologically active metal-containing units with biologically active Lewis bases hoping for some synergistic effect. While this is true for most of the samples presented in this paper it is not true for our efforts with simple diols. This effort was instigated by an observation that even simple diols offered activity against cancer cells. Thus, we synthesized a number of organotin polyethers derived from reaction with various aliphatic and aromatic diols.[43-46] We found that a series of organotin polyethers derived from simple aliphatic diols showed good inhibition of Balb 3T3 cells.[47,48] For instance, the product from dibutyltin dichloride and 1,6-hexanediol showed an EC_{50} value of 5 microg/mL. The product from 1,4-butanediol and dibutyltin dichloride showed an EC_{50}. of 0.25 microg/mL. And finally, the product of dibutyltin dichloride and 1,4-butenediol showed an EC_{50} value of 0.025 microg/mL, the lowest GI_{50} found for organotin products at that time. For comparison, the EC_{50} for cisplatin, the most widely used anticancer chemo drug, is 0.4 microg/mL for this cell line.

This work suggested two structural windows that merited further investigation. These structural windows are that the activity increases as the distance between the oxygen atoms in the diols decreases. Second, that unsaturation, that is the presence of a pi bond in the diol, may contribute to the ability of the organotin polyether to inhibit cell growth. This resulted in the study of a number of organotin polyethers with the results for the PC-3 included in the present study (Tables 16-18).

Table 16 contains results looking at the ability to inhibit cell growth for a series of diols containing terminal hydroxyl groups (Table 16 entries 1-7). In general the EC_{50} decreases as the length of

the distance between the oxygen atoms increases from 1.4 micrograms/mL for ethylene glycol to 0.25 micrograms/mL for 1,8-octanediol. Counter CI_{50} generally decreases as the length between the oxygen atoms increases. Thus, there is a general inverse effect, with the EC_{50} showing inhibition at a lower level as the oxygen atom distance increases while the CI_{50} measure decreases as the distance increases. We have no explanation for this indirect dependency. It must be noted that while there is this indirect dependency, the differences are mild.

Table 16 also contains results as the number of ethylene oxide units are increased (Table 16 entries 1, 8-13). Entries 11-13 are derived from reaction of dibutyltin dichloride with hydroxyl-capped polyethylene glycol (also called polyethylene oxide). These products represent the first reported water soluble organotin polymers and are significant since they allow ready use as a medication through any of the traditional routes. In general, for most of the cancer cell lines studied, these water-soluble polymers exhibit good activity but for the prostate cell line they offer only moderate ability to inhibit cancer growth. For entries 1,8-10 there is a general decrease in EC_{50} and CI_{50} as the distance between the organotin moiety increases. The trend with respect to EC_{50} is counter to what one might think. As the distance between the connective oxygen increases the organotin content decreases so that if the organotin moiety were the main deciding factor, then it would be expected that the ability to inhibit cell growth would decrease, not increase (lower EC_{50} values).

The last two entries in Table 16 are for two diols containing unsaturation, triple bonds. In the first case there is a reasonably low EC_{50} value but low CI_{50} value, but in the final entry in the table there is a relatively high EC_{50} and CI_{50} value. This highlights the problem often encountered in choosing between using EC_{50} or CI_{50} values identify good candidates for further studies. A preferred

outcome is to have low EC_{50} and high CI_{50} values or at least have some direct correlation between the two.

Another way to introduce unsaturation into the polymer chain is to employ Lewis bases that are aromatic. Hydroquinone and substituted hydroquinones fulfill this requirement. Table 17 contains results of such a study. The hydroquinones are arranged in increasing electronegativity. There appears to be little correlation between electronegativity and ability to inhibit PC-3 growth. There does appear to be a relationship between the steric nature of the hydroquinone and PC-3 growth such that the ability to inhibit PC-3 growth as measured by both EC_{50} and CI_{50} is decreased as the bulk on the hydroquinone increases. Thus, the tert-butylhydroquinone and 2,5-di-tert-butylhydroquinone polymers show relatively high EC_{50} and low CI_{50} values while the less bulky methylhydroquinone exhibits relatively low EC_{50} and high CI_{50} values. The influence of bulk is also found in comparing the halogen-containing hydroquinones. Thus, the more bulky bromohydroquinone, 2,5-dichlorohydroquinone and tetrachlorohydroquinone products show higher EC_{50} and lower CI_{50} values compared with the chlorohydroquinone polymer. The relationship between bulk and ability to inhibit PC-3 growth is consistent with size being important in the inhibition of the PC-3 cells. This is consistent with the presence of a size constraint for some important step in cancer growth. (The various mechanisms reported for cancer inhibition by organotin materials have been recently reviewed.[10]) Additionally, this steric dependence does not exist with other cancer cell lines tested so is characteristic of the prostate PC-3 cancer cell line.

Table 18 contains values for diols where the distance between the oxygen atoms is small. For all three polymers there are low EC_{50} values and for the first two, high CI_{50} values. Thus, in this

situation, shortening the distance between the oxygen atoms is positive for at least two of the three polyethers.

Another study involved products from 4,6-diaminopyrimidine and substituted analogues. Table 19 contains the results for this group. There does not appear to be a relationship between electronic nature and ability to inhibit cancer growth. 4,6-Diamino-5-nitropyrimidine and 4,6-diamino-2-chloropyrimidine offer the most electron poor rings yet they offer varied EC_{50} and CI_{50} values with the former offering a poor EC_{50} and medium CI_{50} value while the later shows good EC_{50} and poor CI_{50} values. Also, there does not appear to be a steric dependency in this series. Thus, the 4,6-diaminopyrimidine is the least bulky yet one of the higher EC_{50} and average CI_{50} values.

As noted before, there are a number of sites suggested for intrusion of organotins to act to inhibit cancer cell growth, and it is possible that the difference groups studied here, and even within a group, act differently to inhibit cancer cell growth.[10]

If the steric nature of the Lewis base is important then it should be observed in other studies reported here. This appears not to be the case. Among the Lewis bases that contain the least bulk is thiodiglycolic acid and a Lewis base that is among the most bulky is glycyrrhetinic acid and both exhibit similar EC and CI values. Thus, clearly other features are important in determining the overall ability to inhibit prostate cancer growth.

It also appears that the EC_{50} values are most dependent on the nature of the Lewis base and only secondarily dependent on the nature of the organotin. But, it must be remembered that many of the Lewis bases themselves exhibit little or no biological activity, over the concentration range studied, so the presence of the organotin is critical to the materials exhibiting an ability to inhibit

cell growth. Further, it appears that the presence of biologically active Lewis bases is important in the level of EC_{50} since the values are generally a power of ten greater for the simple diols (Tables 15-18) compared with polymers containing biologically active Lewis bases (Tables 1-12). This is not true for CI_{50} values where both polymers containing biologically active Lewis bases and those containing the simple diols both have instances where CI_{50} values of two and greater are found.

4. Summary

In general, all of the polymers containing biologically active and simple diols exhibit an ability to inhibit PC-3 prostate cancer cells. The concentration level for EC_{50} generally depends largely on the nature of the Lewis base and is only secondarily dependent on the nature of the organotin. For the series of polymers derived from hydroquinone and hydroquinone derivatives, generally the greater the streic nature of the substituent(s) on the hydroquinone the greater the EC_{50} value and poorer the ability of the polymer to inhibit PC-3 cancer cell growth. For CI_{50} values, there does not appear to be a relationship between whether or not the Lewis base is biologically active.

Cell line testing has started, but this needs to continue to more firmly establish structure-property relationships. Most of all, live animal testing must be carried out to see how valid the cell line results are with respect to inhibiting cancer growth within living subjects. This is being planned.

References

1. R. Siegel, CA Cancer J. Clin. **61**, 212 (2011)

2. P. D. Baade, D. R. Youlden, L. J. Krnjacki, Mol. Nutrition Food Res. **53**, 171 (2009)

3. Drugs Approved for Prostate Cancer, 2/11/2014, National Cancer Institute

4. R. E. Sobel, M. D. Sadar, J. Urology **173**, 342 (2005)

5. S. Tai, Y. Sun, J. M. Squires, H. Zhang, W. Oh, C-Z. Liang, J. Huang, Prostate **71**, 1668 ((2011)

6. X-Q. Zhang, X-F. Huang, S-J. Mu, Q-X. An, A-J. Xia, R. Chen, D-C. Wu, Asian J. Androl **12**, 390 (2010)

7. C. Carraher, *Macromolecules Containing Metal and Metal-Like Elements. Vol. 4. Group IVA Polymers*, Wiley, Hoboken, NJ, 2005, pp. 263-310.

8. C. Carraher, M. Roner, S. Shahi, Barot, Materials, **2011**, 4, 801-815.

9. C. Carraher, M. Roner, M., Materials, **2009**, 2**,** 1558-1598.

10. C. Carraher, M. Roner, J. Organomet. Chem. **2014**, 751,67-82.

11. C. Carraher, Y. Naoshima, K. Nagao, Y. Mori, A. Zhao, G. Barot, A. Battin, in Inorganic and Organometallic Macromolecules, Springer, NY, pg 421

12. C. Carraher, M. Roner, K. Shahi, A. Battin, G. Barot, T. Arnold, J. Polym. Mater. **31**, 1 (2014)

13. C. Nebbia, M. Dacasto, L. Ceppa, M. Gennaro Soffietti, P. Spinelli, V. Bergo, P. Di Simplicio, Vet. Res. Commun. **21**, 117 (1997)

14. D. Siegmann-Louda, C. Carraher, Macromolecules Containing Metal and Metal-Like Elements. Vol. 3. Biomedical Applications,, Wiley, Hobokin, 2004.

15. Y. Luo, G.D. Prestwich, Cancer-targeted polymeric drugs, Curr. Cancer Drug Targets **2**, 209 (2002)

16. H. Maeda, Bioconjug. Chem. **21,** 797 (2010)

17. J. Fang, H. Nakamura, H. Maeda, Adv. Drug Deliv. Rev. **63**, 136 (2011)

18. V. Torchilin, Adv. Drug Deliv. Rev. **63**, 131 (2011)

19. P. Goddard, I. Williamson, J. Brown, L.E. Hutchinson, J. Nicholls, K. Petrak, J. Bioact. Compat. Pol. **6**, 4 (1991)

20. P.C. Phillips, Neurologic Clinics, **9**, 383 (1991) 383

21. D.W. Siegmann-Louda, C.Carraher, F. Pflueger, J. Coleman, S. Harless, H. Luing, Polym. Mater. Sci. Eng. **82**, 83 (2000)

22. K. Abdellaoui, M. Boustta, H. Morjani, M. Manfait, M. Vert, J. Drug Target **5**, 193 (1998)

23. P.A. Vasey, S.B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A.H. Thomson, L.S. Murray, T.E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, J. Cassidy, Clin. Cancer Res. **5**, 83 (1999)

24. M.K. Chourasia, S.K. Jain, Drug Deliv. **11**, 129 (2004)

25. E. Fournier, C. Passirani, N. Colin, P. Breton, S. Sagodira, J.P. Benoit, Eur. J. Pharm. Biopharm. **57**, 189 (2004)

26. K. Ulbrich, V. Subr, Adv. Drug Deliv. Rev. **56** 1023 (2004)

27. C. Carraher, R. Chrichton, M. Roner, Polym. Mater. , in press

28. C. Carraher, A. Campbell, M. Roner, Polym. Mater. Sci. Eng., in press

29. C. Carraher, M. Ayoub., M. Roner, A. Moric, N. Trang, J. Chinese Adv. Maters., **1**,65 (2013)

30. C. Carraher, V. Suresh, M. Roner, Polym. Mater. Sci. Eng., in press

31. C. Carraher, N. Sookedo, J. Johnson, Roner, M., Polym. Mater. Sci. Eng., in press

32. C. Carraher, M. Roner, Y. Ashida, G. Barot, J. Polym. Mater., **24**, 357 (2007)

33. C. Carraher, M. Roner, K. Shahi, G. Barot, J. Inorg. Organometal. Polym., **18**, 188 (2008)

34. C. Carraher, Y. Ashida, G. Barot, Polym. Mater. Sci. Eng., **101**, 1405 (2009)

35. C. Carraher, N. Truong, M. Roner, A. Moric-Johnson, N. Sookdeo, N. Trang, Inorg. Chim. Acta, in press

36. C. Carraher, N. Truong, M. Roner, Polym. Mater. Sci. Eng., **105**, 987 (2011)

37. C. Carraher, T. Arnold, M. Roner, Polym. Mater. Sci. Eng., **105**, 990 (2011)

38. M. Roner, J. Williams-Sheffler, C. Carraher, Polym. Mater. Sci. Eng., **102**, 77 (2010)

39. C. Carraher, R. Lambert, M. Roner, Polym. Mater. Sci. Eng., **105**, 983 (2011)

40. C. Carraher, N. Pham, M. Roner, Polym. Mater. Sci. Eng., 107, 345 (2012)

41. G, Barot, C. Carraher, Polym. Mater. Sci. Eng., **98**, 387 (2008)

42. C. Carraher, G. Barot, S. Vetter, G. Nayak, M. Roner, JCAMS **1**, 1(2013)

43. C. Carraher, J. L. Lee, Polym. Mater. Sci. Eng., **90**, 408 (2004)

44. C. Carraher, G. Scherubel, Makromolekular Chemie, **152**, 61 (1972)

45. C. Carraher, G. Scherubel, Makromolekular Chemie, **160**, 259 (1972)

46. C. Carraher, G. Scherubel, J. Polym. Sci., A-1, **9**, 983 (1971)

47. D. Siegmann-Louda, C. Carraher, D. Nagy, D. Snedden, J. Rosa, Polym. Mater. Sci. Eng., **89**, 487 (2003)

48. G. Barot, D. Siegmann-Louda, E. Zevalias, C. Carraher, Polym. Mater. Sci. Eng., **94**, 561 (2006).

49. G. Barot, K. Shahi, M. Roner, C. Carraher, J. Inorg. Organometal. Polym., **17**, 595 (2007)

50. C. Carraher, G. Barot, K. Shahi, M. Roner, J. Chinese Adv. Maters., **1**, 294 (2013)

51. C. Carraher, M. roner, K. Shahi, A. Battin, G. Barot, T. Arnold, J. Polym. Mater., **31**, 1 (2014)

52. C. Carraher, G. Barot, M. Roner, K. Shahi, C. Pellerito, T. Fiore, M. Scopelito, L. Pellerito, J. Polym. Mater., **29**, 361 (2012)

53. C. Carraher, G. Barot, K. Shahi, M. Roner, J. Polym. Mater., **23**, 493 (2006)

54. M. Roner, K. Shahi, G. Barot, A. Batton, C. Carraher, J. Inorg. Organomet. Polym., **19**, 410 (2009)

55. G. Barot, M. Roner, Y. Naoshima, K. Nagao, K. Shahi, C. Carraher, J. Inorg. Organomet. Polym., **19**, 12 (2009)

56. K. Shahi, M. Roner, G. Barot, C. Carraher, Polym. Mater. Sci. Eng., **99**, 349 (2008)

57. Batton, A., K. Shahi, M. Roner, C. Carraher, J. Inor. Organometal. Polym., **17**, 631 (2007)

Tables & Figures (Coupled for easy association between the data and structure)

Table 1 Results for the product of various organotin dihalides with 3-amino-1,2,4-triazole[27]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	3-Amino-1,2,4-triazole	8.0×10^4	21000(2100)	20000(2100)	1.1
Et_2SnCl_2	3-Amino-1,2,4-triazole	4.2×10^5	21000(2100)	20000(2000)	1.1
Bu_2SnCl_2	3-Amino-1,2,4-triazole	3.8×10^4	26000(2500)	23000(2200)	1.1

$Oc_2Sn\ Cl_2$	3-Amino-1,2,4-triazole	1.4×10^5	25000(2400)	12000(1200)	**2.1**
$Ph_2Sn\ Cl_2$	3-Amino-1,2,4-triazole	2.1×10^5	9500(1000)	17000(1900	0.56

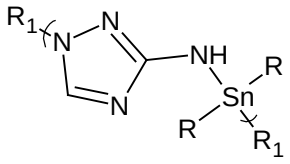


Fig. 1 Repeat unit for the product of organotin dihalides and 3-amino-1,2,4-triazole where R_1 represents chain extension.

Table 2 Results for the product of various organotin dihalides with camphoric acid[28]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Me_2SnCl_2	Camphoric acid	8.0×10^4	54(6)	64(7)	0.84
Et_2SnCl_2	Camphoric acid	4.2×10^5	540(5)	81(7)	6.7
Bu_2SnCl_2	Camphoric acid	3.8×10^4	55(5)	74(6)	0.74
$Oc_2Sn\ Cl_2$	Camphoric acid	1.4×10^5	43(4)	56(6)	0.76
$Ph_2Sn\ Cl_2$	Camphoric acid	2.1×10^5	54(5)	55(5)	0.98
	Camphoric acid itself		1100(10)	1100(12)	1

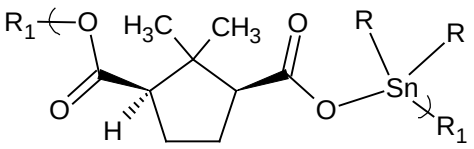


Fig. 2 Repeat unit for the product of camphoric acid and diorganotin dihalides where R represents simple chain extension.

Table 3 Results for the product of various organotin dihalides with chelidonic acid[29]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	Chelidonic Acid	2.0×10^4	>32000	>32000	
Et_2SnCl_2	Chelidonic Acid	1.1×10^4	190(10)	3400(28)	0.12
Bu_2SnCl_2	Chelidonic Acid	1.7×10^5	34(6)	230(6)	0.65
Cy_2SnBr_2	Chelidonic Acid	3.8×10^5	0.34(0.1)	73(6)	0.096
$Oc_2Sn\ Cl_2$	Chelidonic Acid	2.3×10^5	6000(220)	9300(100)	0.49
$Ph_2Sn\ Cl_2$	Chelidonic Acid	1.1×10^5	0.11(0.06)	180(8)	0.002

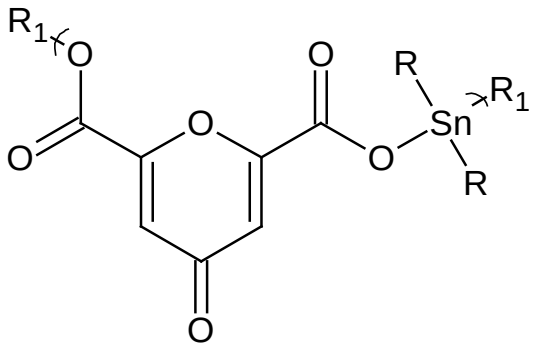


Fig. 3 Repeat unit for the product of organotin dihalides and chelidonic acid where R_1 represents chain extension.

Table 4 Results for the product of various organotin dihalides with cyano-4-hydroxycinnamic acid[30]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Me_2SnCl_2	Cyano-4-hydroxycinnamic acid	8.0×10^4	35000(3500)	39000(3900)	0.90
Et_2SnCl_2	Cyano-4-hydroxycinnamic acid	4.2×10^5	17000(1500)	20000(200)	0.85

Bu_2SnCl_2	Cyano-4-hydroxycinnamic acid	3.8×10^4	26000(2600)	30000(3000)	0.87
Oc_2SnCl_2	Cyano-4-hydroxycinnamic acid	1.4×10^5	20000(2000)	22000(2200)	0.91
Ph_2SnCl_2	Cyano-4-hydroxycinnamic acid	2.1×10^5	16000(1500)	20000(2000)	0.80

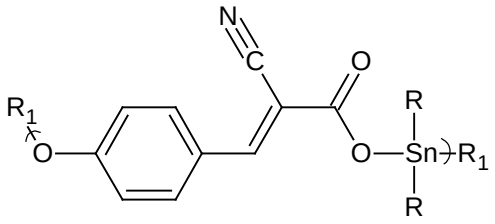


Fig. 4 Repeat unit for the product of organotin dihalides and cyano-4-hydrocinnamic acid where R_1 represents chain extension.

Table 5 Results for the product of various organotin dihalides with dicumarol[31]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**

Me_2SnCl_2	Dicumarol	7.3×10^4	10000(1100)	11000(1100)	0.91
Et_2SnCl_2	Dicumarol	1.9×10^4	20000(2000)	21000(0.95)	0.95
Bu_2SnCl_2	Dicumarol	4.0×10^4	9900(1000)	10000(1000)	0.99
Cy_2SnBr_2	Dicumarol	2.3×10^5	11000(1100)	11000(1100)	1
$Oc_2Sn\ Cl_2$	Dicumarol	1.6×10^5	11000(1100)	15000(1200)	0.72
$Ph_2Sn\ Cl_2$	Dicumarol	2.2×10^4	11000(1000)	11000(1100)	1

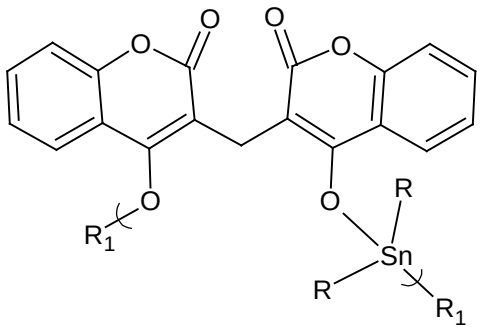


Fig. 5 Repeat unit for the product of organotin dihalides and dicumarol where R_1 represents chain extension.

Table 6 Results for the product of various organotin dihalides with diethylstilbestrol[32,33]

Lewis Acid				Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	75	-1.1	$6.4x10^4$	Diethylstilbestrol	6.4×10^4	1600 (500)	530(500)	**3.0**

Et_2SnCl_2	Diethylstilbestrol	2.1×10^4	50(10)	90(10)	0.56
Pr_2SnCl_2	Diethylstilbestrol	1.2×10^4	2300 (500)	810(50)	**2.8**
Bu_2SnCl_2	Diethylstilbestrol	1.1×10^4	2500 (500)	750(50)	**3.4**
Cy_2SnBr_2	Diethylstilbestrol	9.3×10^3	220(20)	100(10)	**2.2**
Ph_2SnCl_2	Diethylstilbestrol	1.1×10^5	2300 (200)	850(50)	**2.7**
	Diethylstilbestrol		0.25(0.2)	0.67(0.05)	0.37

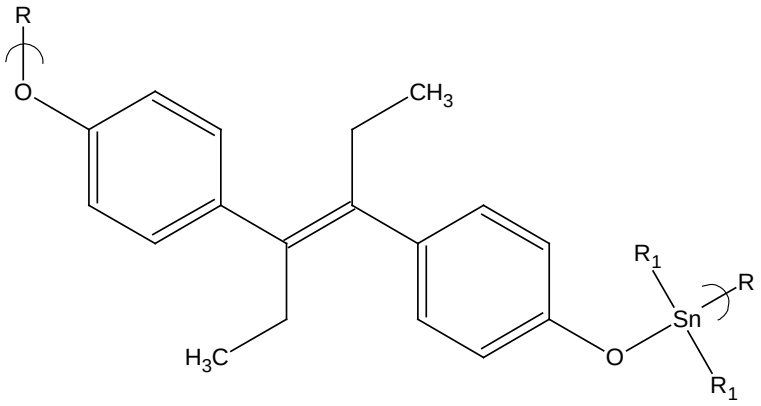


Fig. 6 Repeat unit for the product of organotin dihalides and diethylstilbestrol where R_1 represents chain extension.

Table 7 Results for the product of various organotin dihalides with dienestrol[34]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	Dienestrol	1.3×10^6	1500(500)	810(60)	1.9
Et_2SnCl_2	Dienestrol	1.8×10^6	1400(500)	680(50)	**2.1**
Pr_2SnCl_2	Dienestrol	2.6×10^6	310(200)	680(60)	0.5
Bu_2SnCl_2	Dienestrol	1.3×10^6	60(10)	770(50)	0.1
Cy_2SnBr_2	Dienestrol	1.4×10^6	260(200)	310(40)	0.8

Ph_2SnCl_2	Dienestrol	2.4 x 10^6	190(20)	280(40)	0.7
	Dienestrol		250(200)	660(50)	0.4

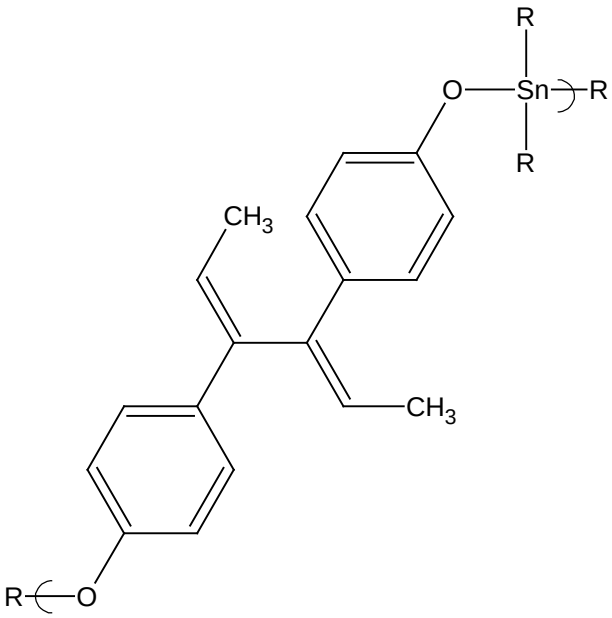


Fig. 7 Repeat unit for the product of organotin dihalides and dienestrol where R_1 represents chain extension.

Table 8 Results for the product of various organotin dihalides with glycyrrhetinic acid[35,36]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**

Me_2SnCl_2	Glycyrrhetinic Acid	7.3×10^5	0.0011(0.001)	0.022(0.01)	0.05
Et_2SnCl_2	Glycyrrhetinic Acid	1.0×10^4	0.0045(0.001)	24(2)	0.0002
Bu_2SnCl_2	Glycyrrhetinic Acid	1.5×10^5	0.0035(0.001)	1.4(0.1)	0.04
Cy_2SnBr_2	Glycyrrhetinic Acid	5.3×10^4	0.16(0.1)	11(2)	0.01
Ph_2SnCl_2	Glycyrrhetinic Acid	2.0×10^4	0.0010(0.001)	1.4(0.2)	0.001

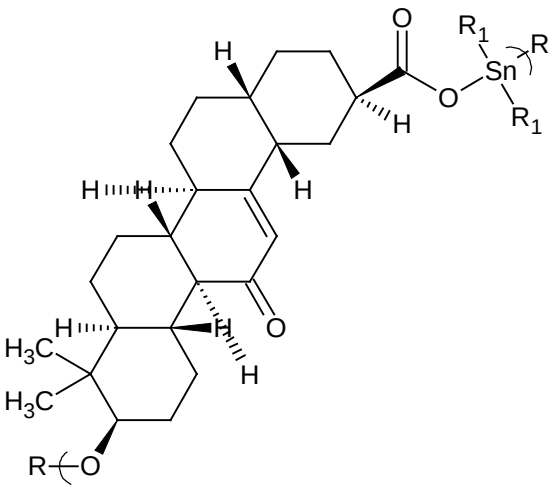


Fig. 8 Repeat unit for the product of organotin dihalides and glycyrrhetinic acid where R_1 represents chain extension.

Table 9 Results for the product of various organotin dihalides with isomannide[37]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**

Me_2SnCl_2	Isomannide	2.6×10^4	0.0034(0.001)	.0036(0.001)	0.94
Et_2SnCl_2	Isomannide	4.4×10^4	>60,000	>60,000	
Bu_2SnCl_2	Isomannide	2.2×10^4	>60,000	>60,000	
Cy_2SnBr_2	Isomannide	1.1×10^5	0.01(0.01)	9.45((1.)	0.001
Ph_2SnCl_2	Isomannide	5.7×10^6	>60,000	>60,000	

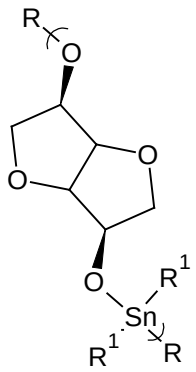


Fig. 9 Repeat unit for the product of organotin dihalides and isomannide where R_1 represents chain extension.

Table 10 Results for the product of various organotin dihalides with phentolamine (Regitine)[38]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**

Me_2SnCl_2	Phentolamine	1.4×10^5	250(100)	780(100)	0.34
Et_2SnCl_2	Phentolamine	4.2×10^4	200(100)	580(100)	0.33
Bu_2SnCl_2	Phentolamine	1.5×10^5	250(100)	730(100)	0.34
Ph_2SnCl_2	Phentolamine	2.2×10^5	200(100)	570(100)	0.57

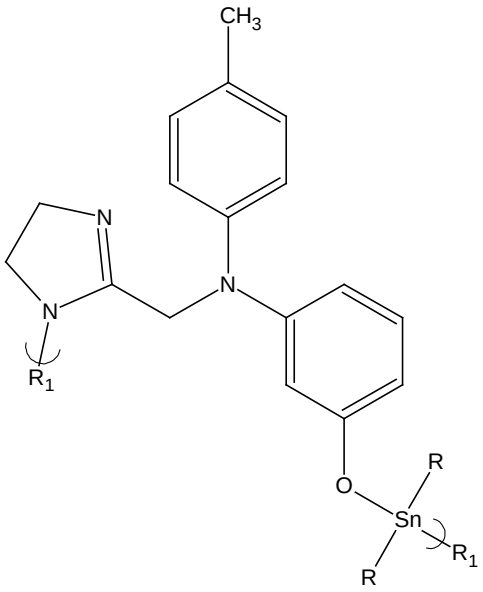


Fig. 10 Repeat unit for the product of organotin dihalides and phentolamine where R_1 represents chain extension.

Table 11 Results for the product of various organotin dihalides with thiamine[39]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	Thiamine	1.4×10^5	500(100)	650(100)	0.77
Et_2SnCl_2	Thiamine	4.2×10^5	550(100)	430(100)	1.3
Bu_2SnCl_2	Thiamine	1.5×10^5	450(100)	550(100)	0.82
Ph_2SnCl_2	Thiamine	2.2×10^5	450(100)	330(100)	1.4

Fig. 11 Repeat unit for the product of organotin dihalides and thiamine where R_1 represents chain extension.

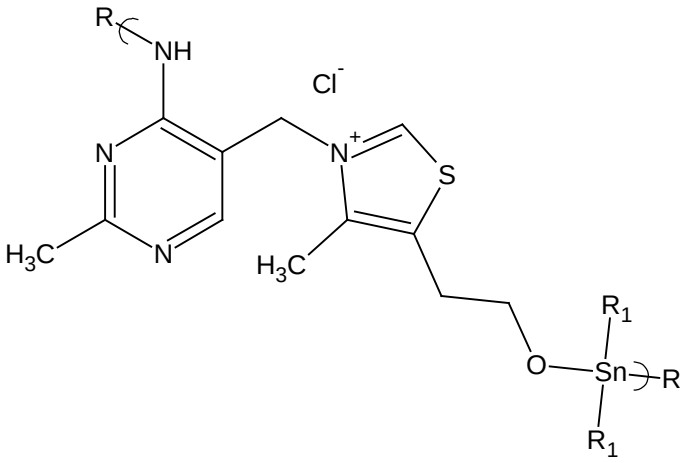


Fig. 12 Repeat unit for the product of organotin dihalides and thiamine where R represents chain extension.

Table 12 Results for the product of various organotin dihalides with thiodiglycolic acid[40]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Me_2Sn	Thiodiglycolic Acid	7.1×10^4	1600(120)	>32000	<0.097
Et_2Sn	Thiodiglycolic Acid	2.9×10^5	440(48)	1,800(8)	0.39
Bu_2Sn	Thiodiglycolic Acid	1.3×10^5	12(6)	56(4)	0.21
Cy_2Sn	Thiodiglycolic Acid	1.1×10^4	0.11(0.09)	16(4)	0.04
Ph_2Sn	Thiodiglycolic Acid	5.0×10^5	3.3(2.4)	190(4)	0.06

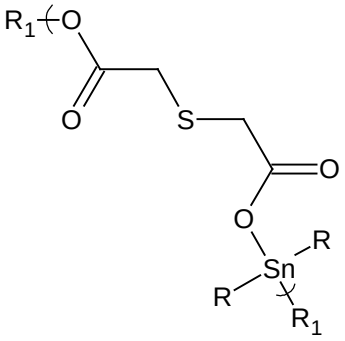


Fig. 12 Repeat unit for the product of organotin dihalides and thiodiglycolic acid where R_1 represents chain extension.

Table 13 Inhibition results for the organotin monomers

Lewis Acid	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Me_2SnCl_2	220(100)	510(100)	0.43
Et_2SnCl_2	200(100)	610(100)	0.33
Pr_2SnCl_2	250(100)	380(100)	0.66
Bu_2SnCl_2	200(50)	1400(100)	0.14
Ph_2SnCl_2	250(100)	820(100)	0.30
Cy_2SnCl_2	200(100)	480(100)	0.42
Oc_2SnCl_2	300(100)	550(100)	0.56
Bz_2SnCl_2	200(100)	900(100)	0.22
Cisplatin	50(40)	1000(100)	0.05

Table 14 Best EC_{50} and CI_{50} values for each Lewis base group.

Lewis Base	Lowest EC_{50}	Highest CI_{50}

3-Amino-1,2,4-triazole	Oc_2Sn 12000	Oc_2Sn 2.1
Camphoric acid	Ph_2Sn 55	Et_2Sn 6.7
Chelidonic acid	Cy_2Sn 73	Bu_2Sn 0.65
Cyano-4-hydroxycinnamic acid	Ph_2Sn 20000	Oc_2Sn 0.91
Dicumarol	Bu_2Sn 10000	Ph_2Sn 1; Cy_2Sn 1
DES	Et_2Sn 90; Cy_2Sn 100	Bu_2Sn 3.4; Me_2Sn 3.0
Dienestrol	Ph_2Sn 0.28; Cy_2Sn 0.31	Et_2Sn 2.1
Glycyrrhetinic acid	Me_2Sn 0.022	Me_2Sn 0.05
Isomannide	Me_2Sn 0.0036	Me_2Sn 0.05
Phentolamine	Ph_2Sn 570; Et_2Sn 580	Ph_2Sn 0.57
Thiamine	Ph_2Sn 330	Ph_2Sn 1.4; Et_2Sn 1.3
Thiodiglycolic acid	Cy_2Sn 16	Et_2Sn 0.39
Cisplatin	1000	0.05

Table 15 Inhibition results as a function of chain length.[41]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Bu_2SnCl_2	1,5-Pentanediol	1.8×10^4	50(10)	300(20)	0.17
Bu_2SnCl_2	1,5-Pentanediol	1.6×10^4	80(10)	2200(200)	0.037
Bu_2SnCl_2	1,5-Pentanediol	1.3×10^4	870(20)	2200(200)	0.040
Bu_2SnCl_2	1,5-Pentanediol	1.06×10^4	230(30)	2700(300)	0.086
Bu_2SnCl_2	1,3-Propanediol	3.0×10^4	50(10)	440(40)	0.11
Bu_2SnCl_2	1,3-Propanediol	2.45×10^4	170(30)	780(60)	0.21
Bu_2SnCl_2	1,3-Propanediol	2.07×10^4	46(10)	770(60)	0.06
Bu_2SnCl_2	1,3-Propanediol	1.74×10^4	50(10)	730(60)	0.06

Table 16 Cell growth for dibutyltin polyethers.[49-54]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} PC-3	CI_{50}
Bu_2SnCl_2	Ethylene Glycol	1.0×10^4	900(100)	1400(100)	1.2
Bu_2SnCl_2	1,3-Propanediol	3.0×10^4	50(10)	440(40)	0.05

Bu_2SnCl_2	1,4-Butanediol		60(10)	120(10)	0.21
Bu_2SnCl_2	1,5-Pentandeiol	1.8×10^4	50(10)	300(20)	0.23
Bu_2SnCl_2	1,6-Hexanediol	2.1×10^5	50(10)	1000(150)	0.36
Bu_2SnCl_2	1,7-Heptanediol	1.2×10^4	40(10)	250(20)	0.07
Bu_2SnCl_2	1,8-Octanediol	4.0×10^4	20(10)	210(20)	0.07
Bu_2SnCl_2	Diethylene Glycol	1.1×10^5	1200(100)	900(100)	1.3
Bu_2SnCl_2	Triethylene Glycol	1.7×10^5	1100(100)	1000(100)	1.1
Bu_2SnCl_2	Pentaethylene Glycol	4.7×10^5	50(10)	600(50)	0.006
Bu_2SnCl_2	PEG-400	7.6×10^4	280(30)	2100(200)	0.13
Bu_2SnCl_2	PEG-8,000	1.1×10^5	1000(100)	250(10)	0.13
Bu_2SnCl_2	PEH-10,000	7.6×10^4	1000(100)	10000(1000)	0.10
Bu_2SnCl_2	2,5-Dimethyl-6-hexyne-2,5-diol	1.35×10^5	210(100)	320(100)	0.66
Bu_2SnCl_2	2-Butyne-1,4-diol	1.2×10^5	3000(280)	1300(100)	**2.3**

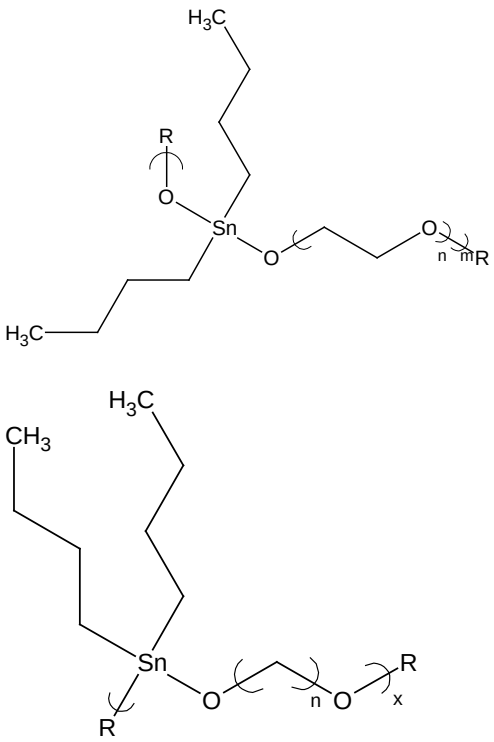


Fig. 13 Repeat unit for the polyethers derived from reaction of dibutyltin dichloride and various hydroxyl-terminated ethylene oxides (right) and methylene spacer diols (left) where R represents chain extension.

Table 17 Yield and molecular weight data for the dibutyltin polyethers derived from hydroquinone and the hydroquinone derivatives [55]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Bu_2SnCl_2	Methoxyhydroquinone	4.2×10^4	2000(300)	280(100)	**7.0**
Bu_2SnCl_2	Tert-Butylhydroquinone	3.3×10^4	1800(500)	2500(200)	0.71
Bu_2SnCl_2	2,5-Di-tert-Butylhydroquinone	2.0×10^4	2200(500)	2600(200)	0.84
Bu_2SnCl_2	Methylhydroquinone	2.9×10^4	2600(500)	300(100)	**8.5**
Bu_2SnCl_2	Phenylhydroquinone	3.3×10^4	210(30)	280(100)	0.75
Bu_2SnCl_2	Hydroquinone	2.2×10^4	2000(500)	440(100)	**4.4**
Bu_2SnCl_2	2,3-Dicyanohydroquinone	8.3×10^4	2400(500)	2600(200)	0.9
Bu_2SnCl_2	Bromphydroquinone	2.6×10^4	250(100)	2500(200)	0.1
Bu_2SnCl_2	Chlorohydroquinone	5.9×10^4	10000(0300)	2200(100)	**4.6**
Bu_2SnCl_2	2,5-Dichlorohydroquinone	2.5×10^4	1900(300)	2800(200)	0.67
Bu_2SnCl_2	Tetrachlorohydroquinone	1.9×10^5	2200(500)	6500(200)	0.34
Bu_2SnCl_2	2,5-Dihydroxybenzaldehyde	6.8×10^4	2000(500)	2500(200)	0.58

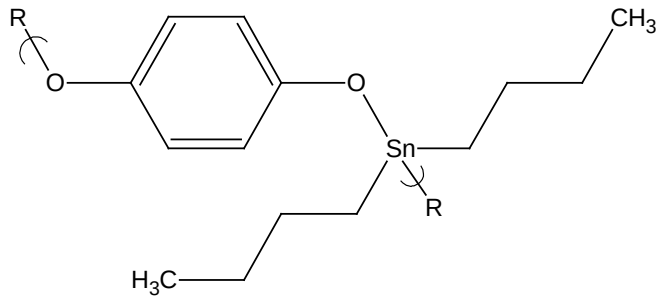


Fig. 14 Repeat unit for the product of organotin dihalides and hydroquinone where R represents chain extension.

Table 18 Product biological activities for organotin polyethers derived from 1,1- and 1,2-diols [56]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Bu_2SnCl_2	2,3-Butanediol	5.6×10^4	1100(300)	290(100)	**3.8**
Bu_2SnCl_2	3-Chloro-1,2-propanediol	4.5×10^4	1900(400)	201(100)	**9.0**

Bu_2SnCl_2	Neopentyle Glycol	8.1×10^4	150(100)	180(80)	0.80

Table 19 Inhibition results for dibutyltin dichloride polyamines derived from 4,6-diaminopyrimidine and substituted 4,6-diaminopyrimidines [57]

Lewis Acid	**Diamine**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} PC-3**	**CI_{50}**
Bu_2SnCl_2	4,6-Diaminopyrimidine	$3.7x10^6$	1100(100)	1300(100)	0.85
Bu_2SnCl_2	4,6-Diamino-5-nitro-pyrimidine	1.3×10^5	1100(100)	1100(100)	1.00
Bu_2SnCl_2	4,6-Diamino-2-methyl-mercaptopyrimidine	1.8×10^5	1400(100)	1100(100)	1.3
Bu_2SnCl_2	4,6-Diamino-2-methyl-5-nitrosopyrimidine	5.5×10^5	1400(100)	900(100)	1.6
Bu_2SnCl_2	4,6-Diamino-2-mercaptopyrimidine	1.8×10^6	250(10)	400(40)	0.53
Bu_2SnCl_2	4,6-Diamino-2-chloropyrimidine	1.4×10^5	40(30)	400(40)	0.10

Bu_2SnCl_2	4,6-Diamino-2-hydroxylpyrimidine	2.1×10^5	50(10)	410(040)	0.12
Bu_2SnCl_2	4,6-Diamino-1-nitroso-2-hydroxylpyrimidine	2.8×10^5	80(10)	40(40)	0.18
Bu_2SnCl_2	4,6-Diamino-5-(4-chloro-phenyl)-6-ethylpyrimidine	3.5×10^4	250(30)	750(40)	0.33

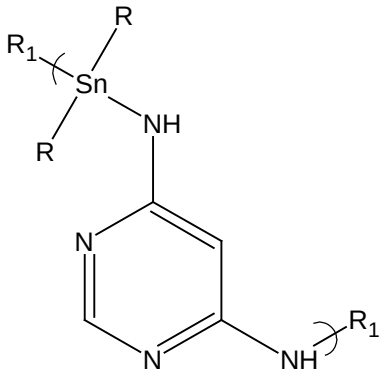


Fig. 15 Repeat unit for the product of organotin dihalides and 4,6-diaminopyrimidine where R_1 represents chain extension.

CHAPTER 5

CONTROL OF BREAST CANCER USING ORGANOTIN POLYMERS[3]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 1-20) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher, Charles E., Michael R. Roner, Kimberly Shahi, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot, Amitabh Battin, Nancy T. Trang, Nandalall Sookdeo, and Zamil Islam

Carraher, Charles E., Michael R. Roner, Kimberly Shahi, Alisa Moric-Johnson, **Lindsey Miller**, Girish Barot, Amitabh Battin, Nancy T. Trang, Nandalall Sookdeo, and Zamil Islam (**2015**). "Control of Breast Cancer Using Organotin Polymers." International Journal of Polymeric Materials and Polymeric Biomaterials 64.15, 800-14.

[3] Used with permission of Taylor & Francis, 2017

Control of Breast Cancer Using Organotin Polymers

Charles E. Carraher Jr., [a,b] Michael R. Roner,[c] Kimberly Shahi,[c] Alisa Moric-Johnson,[c] Lindsey Miller,[c] Girish Barot,[a] Amitabh Battin,[a] Nancy T. Trang,[c] Mohammed Harb Alhuniti,[a] Zamil Islam [a]

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

b. Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410 (carraher@fau.edu**)**

c. University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Abstract The efforts described in this paper are aimed at designing organotin polymers that control the growth of breast cancer and to identify structure/property relationships that will assist in this goal. The growth of MCF-7 and MDA-MB-231 breast cancer cell lines is inhibited employing a wide range of organotin condensation polymers. The EC_{50} values are primarily dependant on the nature of the Lewis base but the CI_{50} is dependent on both the nature of the Lewis base and Lewis acid. A number of products exhibit CI_{50} values greater than two. These include a number of organotin polyethers such as those derived from diethylstilbestrol, dienestrol, short-chained dibutyltin polyethers, and hydroquinone derivatives. In each of these cases the MDA-MB-231 cells exhibit greater inhibition compared to the estrogen receptor (ER) MCF-7 cells. The organotin polymers generally exhibit a superior ability to inhibit MCF-7 and MDA-MB-231cell growth compared to the standard cisplatin.

Keywords Organotin polymers, tin-containing polymer, breast cancer, MCF-7 cell line, MDA-MB-231 cell line, interfacial polycondensation, anticancer drugs

Running title Control Breast Cancer with Organotin Polymers

1Introduction

1.2 General

Cancer is the leading cause of death globally.[1,2] Breast cancer accounts for about 23% of all cancers (excluding non-melanoma skin cancers) in women. Survival rates depend on a number of factors but the main one is the stage of the cancer.[3,4] The stages run from zero which is a pre-cancerous or marker condition to stage 4 which is metastatic.[3] The survival rate for stages 0 and 1 is about 100% but for stage 4 only 22%. As in most cancers, survival is defined as being a five year survival. Treatments include surgery, hormonal therapy, chemotherapy, immunotherapy, and radiation.[3] Here we will concentrate on chemotherapy. A number of drugs are employed to treat breast cancer.[3] According to the National Cancer Institute these include cisplatin and derivatives, taxol and derivatives, cyclophosphamide, tamoxifen, gemcitabine, fluorouracil, adriamycin and methotrexate.[3]

The objective of our studies is to develop compounds that demonstrate good ability to curtail cancer growth, here breast cancer growth, with minimal harm to the patient. It also involves developing structure/property relationships that will guide further efforts and eventually identify important sites that intersect cancer growth.

The most widely used breast cancer cell lines are the MDA-MB-231 (strain number 7233) cells that are estrogen-independent, estrogen receptor negative while the MCF-7 (strain line 7259) cells are estrogen receptor (ER) positive.[5-7] These two often serve as a pair when looking at the ability of compounds to inhibit breast cancer growth and are the pair employed by us in evaluating the ability of our organotin polymers to inhibit breast cancer. MCF-7 cells were initially isolated in 1970. They are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability of these cells to process estrogen, in the form of estradiol. Thus, MCF-7 cells are an estrogen receptor, ER, positive control cell line. They are also sensitive to cytokeratin, and are unreceptive to desmin, endothelin, vimentin, and GAP. Growth is inhibited using tumor necrosis factor alpha (TNF alpha) and treatment of MCF-7 cells with anti-estrogens often modulate insulin-like growth factor finding protein's often resulting in a reduction of cell growth.

We have synthesized many metal-containing polymers for control of various microbes including bacteria, fungus, virus, and cancer. Much of this has been recently reviewed.[8-13] Here we will focus on tin-containing polymers.

Work employing organotin small molecules as potential anticancer agents has been ongoing for about 80 years with many contributing.[11] These contributions include both the synthesis and testing of the organotin-containing compounds as to their ability to slow the growth of various cancers and preliminary mechanistic studies identifying various sites where the specific organotin compounds appear to intersect cancer growth. These aspects have been recently reviewed.[9-11]

One major approach taken by us is to employ biologically active reactants, incorporating them within polymers often coupling the biologically active metal-containing moiety with a Lewis base that itself offers biological activity hoping for a synergetic effect by having both biologically active moieties within the same polymer chain.

As part of the biological characterization of many of the recently synthesized organotin polymers we included the ability to inhibit a battery of human-cancer cell lines including the MDA-MB-231 (strain number 7233) cells that are estrogen-independent, estrogen receptor negative while the MCF-7 (strain line 7259) cells are estrogen receptor (ER) positive. The current paper examines the results of these studies focusing on identifying the possible influence of certain structural features to inhibit the cancer cell line growth of these two cancer cell lines. It is also the purpose to see if there are differences in cell line inhibition between the two cell lines.

1.2 Advantages of Polymer Drugs

The advantages of polymeric drugs have been recently reviewed emphasizing their use in combating cancer.[9-11,14-19] Following briefly describes some of these advantages in comparison to small, monomeric drugs.

First, polymers should be filtered out by the kidneys more slowly than small compounds[20] decreasing kidney damage and increasing body retention time. This should allow a greater time for the cancer to be attacked by the polymeric drug. Second, a large polymer may be effective against tumors that have developed resistance to other chemotherapeutic agents, either because the polymer is not recognized by cellular resistance mechanisms or because the polymer could be used to deliver local, high-dose chemotherapy.[21] This is particularly important in the treatment of so called resistant cancers that have developed resistance subsequent to initial treatment of by chemo agents. This developed resistance is normal in chemo patients. Our polymers have shown good inhibition of such resistant cancer cell lines.[22] Third, the size and structure of the polymer should provide more binding sites to cellular targets, increasing effectiveness. Fourth, polymers can be designed to incorporate multiple anticancer agents within the same molecule that act against cancer cells by different mechanisms. Our practice of incorporating biologically active Lewis bases along with the organotin moiety has provided a number of polymers with good ability to inhibit cancer cell growth. Fifth, the polymeric structure permits easy coupling to other molecules, such as those that specifically target cancer cells, allowing delivery of a polymeric drug to a particular site without impairing its effectiveness. Sixth, polymers can be designed as either large stable compounds that enter the cell by pinocytosis [23,24] and is active in a polymeric form, or as an unstable compound that degrades slowly into active monomeric units in some timed-release fashion. [25-27] Seventh, large molecules accumulate in solid tumors more than in normal tissues because of the enhanced permeability and retention effect. This results in high amounts of the polymer occupying the interstitial space due to the leaky vasculature and limited lymphatic drainage typical of tumors.[27] This effect is referred to as the enhanced permeability and retention effect, EPR effect.[17-19,27] Finally, polymers can be synthesized to fine-tune structural characteristics, such as monomeric components, chain length, cross-linking, and polarity, to maximize anticancer activity and vary

the spectrum of activity. Thus, polymers offer a wide degree of flexibility in their design and many possible benefits compared to monomeric drugs.

2 Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the Lewis base and sodium hydroxide 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 room temperature, about 25 ^{o}C). Stirring was begun and an organic phase (typically 30 mL), generally heptane and the organotin dihalide, rapidly added through a hole in the jar lid using a powder funnel. Addition generally takes less than 3 seconds. 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.

2.2 Physical Characterization

Molecular weight was obtained using Light scattering photometry employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. While not reported here, the structural determination of the polymers was carried out employing the following. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ^{1}H NMR spectra were obtained in d-6 DMSO employing Varian Inova 400 MHz amd 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 Pro MALDI mass spectrophotometer, Applied Biosystems, Foster City, CA.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human breast adenocarcinoma cell lines MCF-7 and MDA MB-231 and human normal embryonic lung fibroblast (WI-38) cell line used as the standard. Cells were seeded into a 96-well culture plate at a density of 20,000 cells per well in 100 µL of culture medium. Following a 24 h incubation period, the test compounds were added at concentrations ranging from 0.0032 to 32,000 (or 60,000) ng/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 µL /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 cell 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 (or in the case of the water soluble organotin polymers in water) and serial dilutions prepared in MEM to treat the cells, then the mock-treated cells were “treated” with the same serial dilutions of DMSO without added chemotherapeutic compound. This was done to ensure that any cytotoxicity observed was due to the activity of the compound and not the diluents. For the studies reported here, the mock-treatment never resulted in a loss of cell viability

of more than 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.

3 Results and Discussion

3.1 Standard Biological Measures

Different measures of the ability and effectiveness of drugs to arrest cell growth for cell line studies are employed. The two most widely have been employed in our studies. The first measure of the effectiveness to inhibit cell growth is the effective concentration, EC, to cause growth inhibition for some inhibition fraction, usually the concentration to inhibit the growth by 50%, EC_{50}. The second measure of effectiveness is the chemotherapeutic index, CI, again for some fraction generally 50%, CI_{50}. The chemotherapeutic index is the concentration of the compound that inhibits the growth of the normal cells, usually the NIH/3T3 or WI-38 cells, by 50% divided by the concentration of the compound that inhibits the growth of the cancer cells by 50%. Larger values are desired since they indicate that a larger concentration is required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicate some preference for inhibiting the cancer cells in comparison to the normal cells. There is not general agreement as to which of these two values is most meaningful when evaluating cancer inhibition.

The EC_{50} and CI_{50} values for the polymer samples are generally compared to the ability of the monomer and some chemo agent to inhibit cell growth. Cisplatin, among the most widely used chemo agents, is normally employed as the standard chemo agent by us and is employed in the present studies.

3.2 Inhibition Results

Tables 1-13 and 16-20 contain MCF-7 and MDA-MB-231breast cell line results for polymers grouped together according to the particular Lewis base. Each set also has references included that give the original synthesis for these materials. The EC_{50} values are given in nanograms/mL, ng/mL. CI_{50} values of two and greater are considered significant and given in bold. The Lewis bases typically offer little or no inhibition of the MCF-7 and MDA-MB-231 cell lines. Where this is not the case, the inhibition values are included. The repeat unit for each group is given following the appropriate table. Inhibition by the organotin monomers is given in Table 14. Tables 1-14 and 16-20 contain the EC_{50} for the standard, here WI-38 cells, and for the particular compound tested. They also contain the CI_{50} which is the EC_{50} WI-38/EC_{50} MCF-7 or MDA-MB-231 for each of the polymers. The literature citation to the synthesis and physical characterization for each polymer series accompanies the table presenting the data.

Table 15 contains the best (lowest) EC_{50} values and (highest) CI_{50} values for each of the different Lewis base groups. Several observations are apparent. First, the inhibition indicators, EC_{50} and CI_{50} values, vary according to the nature of both the organotin and Lewis base. Second, with much of our effort with organotin-containing polymers, we observed that the most active products contained the dibutyltin moiety. While this was observed for the bank of cancer cells tested by us that included breast, colon, pancreatic, bone, lung, and prostrate cancers, is this also true for the

MCF-7 and MDA-MB-231 cell lines? We see that there is no clear trend with respect to which organotin gives the lowest EC and highest CI values within each Lewis base grouping (Table 15). Thus, the trend of the best values being found for the dibutyltin-containing polymers is not followed for the MCF-7 and MDA-MB-231 results. In comparison to the standard cisplatin, the EC and CI values are generally superior. In fact, with respect to CI_{50} all of the compounds are superior (higher) to cisplatin. Third, the employed Lewis bases offer a broad range of connective groups to the organotin moiety including Sn-O, Sn-N and Sn-O-C(O) as well as mixed linkages. Thus, they represent all of the most common linkages for linear organotin polycondensation polymers. There does not appear to be any linkage that is markedly superior or inferior to inhibiting the MCF-7 and MDA-MB-231 breast cancer cell lines.

In looking at the data from Tables 1-13 there is a wide range of EC_{50} values from >60,000 ng/mL, the highest concentration tested for the polymers, to 1.5 ng/mL for the dimethyltin/isomannide product for the MDA cell line and 1.5 ng/mL for the same pair for the MCF-7 cell line. These values are about 10^3 lower compared to cisplatin. There is also a wide variety of CI_{50} with the highest values being for dibutyltin product with histamine of 670 for the histamine/Bu_2Sn pair for the MDA cell line and 360 for the same pair for the MCF cell line. These CI_{50} are 10^4 greater than for cisplatin for the MDA cell line and 2 x 10^4 times greater than for cisplatin for the MCF cell line. Within a given group designated by a particular Lewis base, the EC_{50} and CI_{50} values are generally similar. Thus, to a first approximation, the ability to inhibit MCF-7 and MDA-MB-231 cancer cells is mostly dependent on the nature of the Lewis base. This is significant since it allows the dibutyltin moiety to be used when needed. The dibutyltin moiety is favored for a number of reasons including of the alkytin moieties it is the least toxic towards humans; it has been employed commercially for over 50 years; it is the most commercially available; and it is the least expensive.[43] Further, in nature it degrades to the relatively nontoxic tin oxide.[43]

As noted before, in most cases, the EC_{50} values are lower than for cisplatin for both cell lines. In general, CI_{50} values of 2 and greater are considered significant. Looking at only the highest value results given in Table 15, there are only 6 out of the possible 13 Lewis base groups that have values of 2 and greater for the MCF cell line and only 4 examples for the MCF cell line. Further, in all cases the CI_{50} values of two and greater listed in Table 13 are higher or equal for the MDA cells compared to the MCF cells. It is interesting that there is not a general agreement between the compounds within a Lewis base grouping with respect to which polymer gives the lowest EC_{50} and the highest Cl_{50} value. This is consistent with the general differences found in the current study as to which measure is more meaningful.

The products derived from histamine deserve special comment. In 11 of twelve opportunities, CI_{50} values are greater than 2 with some greater than 100. Yet the EC_{50} values are modest. The high values for CI_{50} are due to the especially low toxicities, high EC_{50} values, the polymers have against the standard WI-38 cells.

The tables also contain the average molecular weights for each of the polymers. We have found that the ability to inhibit various cancer cell lines is not primarily dependent on chain length (Table 16).[45] We have also found that the active agent is the intact polymer chain .[46]

For the products given in Table 16, as chain length changes presumably changes of not only chain size occurs, but also the particular fraction of end group with a particular end group also changes. Since the EC_{50} and CI_{50} values are similar, ability to inhibit cancer growth appears to be largely independent of both chain length and nature of the end group for a given Lewis acid/base pair. More study is needed before this is more confidently accepted as true.

As noted above, much of our recent effort focused on the coupling of biologically active metal-containing units with biologically active Lewis bases hoping for some synergistic effect. While this is true for most of the samples presented in this paper it is not true for our efforts with simple diols. This effort was instigated by an observation that even simple diols offered activity against cancer cells. Thus, we synthesized a number of organotin polyethers derived from reaction with various aliphatic and aromatic diols.[47-50] We found that a series of organotin polyethers derived from simple aliphatic diols showed good inhibition of Balb 3T3 cells.[51,52] For instance, the product from dibutyltin dichloride and 1,6-hexanediol showed an EC_{50} value of 5 microg/mL. The product from 1,4-butanediol and dibutyltin dichloride showed an EC_{50}. of 0.25 microg/mL. And finally, the product of dibutyltin dichloride and 1,4-butenediol showed an EC_{50} value of 0.025 microg/mL, the lowest GI_{50} found for organotin products at that time. For comparison, the EC_{50} for cisplatin, the most widely used anticancer chemo drug, is 0.4 microg/mL for this cell line.

This work suggested two structural windows that merited further investigation. These structural windows are that the activity increases as the distance between the oxygen atoms in the diols decreases. Second, that unsaturation, that is the presence of a pi bond in the diol, may contribute to the ability of the organotin polyether to inhibit cell growth. This resulted in the study of a number of organotin polyethers with the results for the MCF-7 and MDA-MB-231 included in the present study (Tables 17-19).

Table 17 contains results looking at the ability to inhibit cell growth for a series of diols containing terminal hydroxyl groups (Table 17 entries 1-7). In general the EC_{50} decreases as the length of the distance between the oxygen atoms increases from 300 ngrams/mL for ethylene glycol to 90 nanograms/mL for 1,8-octanediol for the MDA cell line and from 1000 ng/mL for ethylene glycol to 220 ng/mL for the MCF cell line. Counter CI_{50} values generally decreases as the length between the oxygen atoms increases for the MDA cell line and a less direct decrease for the MCF cell line. Thus, there is a general inverse effect, with the EC_{50} showing inhibition at a lower level as the oxygen atom distance increases while the CI_{50} measure decreases as the distance increases. We have no explanation for this indirect dependency. It must be noted that while there is this indirect dependency, the differences are mild once we get beyond the ethylene glycol as the diol.

Table 17 also contains results as the number of ethylene oxide units are increased (Table 17 entries 1, 8-13). Entries 11-13 are derived from reaction of dibutyltin dichloride with hydroxyl-capped polyethylene glycols (also called polyethylene oxides). These products represent the first reported

water soluble organotin polymers and are significant since they allow ready use as a medication through any of the traditional routes. In general, for most of the cancer cell lines studied, these water-soluble polymers exhibit good activity but for the breast cell lines they offer only moderate ability to inhibit cancer growth. For entries 1,8-10, if the results for the ethylene glycol are omitted, there is little difference between the EC_{50} values, but there is a direct decrease in the CI_{50} value as the distance between the organotin moiety increases. The trend with respect to CI_{50} is as expected. As the distance between the connective oxygen increases the organotin content decreases so that if the organotin moiety were the main deciding factor, then it would be expected that the ability to inhibit cell growth would decrease consistent with what is observed.

The last two entries in Table 17 are for two diols containing triple bonds as the site of unsaturation. In the first case there is a reasonably low EC_{50} value but low CI_{50} value, but in the final entry in the table there is a relatively high EC_{50} and CI_{50} value. This highlights the problem often encountered in choosing between using EC_{50} or CI_{50} values identify good candidates for further studies. A preferred outcome is to have low EC_{50} and high CI_{50} values or at least have some direct correlation between the two.

Another way to introduce unsaturation into the polymer chain is to employ Lewis bases that are aromatic. Hydroquinone and substituted hydroquinones fulfill this requirement. Table 18 contains results of such a study. The hydroquinones are arranged in increasing electronegativity. There appears to be little correlation between electronegativity and ability to inhibit the breast cell growth. For prostate PC-3 cells, there a relationship between the steric nature of the hydroquinone derivatives and PC-3 growth such that the ability to inhibit PC-3 growth as measured by both EC_{50} and CI_{50} is decreased as the bulk on the hydroquinone increases. This trend is not found for either breast cancer cell line. The relationship between bulk and ability to inhibit PC-3 growth is in agreement with size being important in the inhibition of the PC-3 cells. This is consistent with the presence of a size constraint for some important step in inhibiting cancer growth. (The various mechanisms reported for cancer inhibition by organotin materials have been recently reviewed.[11]) Since this is not present for the breast cancer cell lines, it is possible that the two cell lines operate to inhibit cancer cell growth by different mechanisms.

One important observation is apparent for the hydroquinone results. Most exhibit high CI_{50} values for the MDA-MB-231 cell line but smaller, generally a power of ten smaller, for the MCF-7 cell line. We previously reported this observation structurally attributing this to the presence of the phenylene-O-Sn moiety.[9,11] A similar distinction is found for the diethylstilbestrol (Table 6) and dienestrol (Table 7) products where inhibition of the MDA-MB-231 is greatly superior to that found for the MCF-7 cancer cell line measured using CI_{50} values. Each of these product-groups contains the phenylene-O-Sn structure. We believe that this is because the MCF-7 cells are estrogen receptor positive and that much of the drug is taken on by the MCF-7 cells instead of allowing the drug to act on the cell line. This is also consistent with the observation reported in the introduction that anti-estrogens can modulate insulin-like growth factor finding protein's acting to reduce cell growth. Both stilbestrol and dienestrol are hormones that act on the MCF-7 estrogen sensitive cells but leaving the MDA-MB-231 cells alone to be inhibited by the full concentration

of the applied drug. It is interesting that a similar result occurs for hydroquinone which has a similar phenylene-O-Sn moiety but this differentiation is not found for the other organotin polymers given in Tables 15 and 16. Neither is this difference found for any of the other polymers presented in this paper (Tables 1-13). But based on EC_{50} values the opposite tendency is found in that lower values are found for the MDA cells compared to the MCF-7 cells. This again points to the difference found between evaluation of ability to inhibit a particular cell growth based on EC_{50} and CI_{50} values.

Table 18 contains values for diols where the distance between the oxygen atoms is small. For all four polymers there are higher CI_{50} values for the MDA-MB-231 cell line compared with the MCF-7 similar to that found for the stilbestrol, dienestrol, and hydroquinone derivatives. Thus, there is probably some structural similarity between the polymers reported on in Table 18 and those described in Tables 6,7 and 18 but not present in Table 16 for other dibutyltin diols. All four of the compounds reported in Table 19 show good CI_{50} values so shortening the distance between the oxygen atoms is positive for at least these polyethers.

Another study involved products from 4,6-diaminopyrimidine and substituted analogues. Table 20 contains the results for this group. There does not appear to be a relationship between electronic nature and ability to inhibit cancer growth. 4,6-Diamino-5-nitropyrimidine and 4,6-diamino-2-chloropyrimidine offer the most electron poor rings yet they offer varied EC_{50} and CI_{50} values with the former offering a poor EC_{50} and medium CI_{50} value while the later shows good EC_{50} and poor CI_{50} values. Also, there does not appear to be a steric dependency in this series. Thus, the 4,6-diaminopyrimidine is the least bulky yet an average EC_{50} and CI_{50} values for both cell lines.

As noted before, there are a number of sites suggested for intrusion of organotins to act to inhibit cancer cell growth, and it is possible that the difference groups studied here, and even within a group, act differently to inhibit cancer cell growth.[11]

If the steric nature of the Lewis base is important as in the prostate PC-3 cell line then it should be observed in other studies reported here. This appears not to be the case. Among the Lewis bases that contain the least bulk is thiodiglycolic acid and a Lewis base that is among the most bulky is glycyrrhetinic acid and both exhibit similar EC_{50} and CI_{50} values. Thus, clearly other features are important in determining the overall ability to inhibit prostate cancer growth.

Table 14 contains values for the organotin monomers. None show particularly low EC_{50} values and none show CI_{50} values greater than one. Thus, while the presence of the organotin moiety is essential to showing some ability to inhibit MCF-7 and MDA-MB-231 growth, none are sufficient to show good MCF-7 and MDA-MB-231 inhibition.

It also appears that the EC_{50} values are most dependent on the nature of the Lewis base and only secondarily dependent on the nature of the organotin. But, it must be remembered that many of the Lewis bases themselves exhibit little or no biological activity, over the concentration range studied, so the presence of the organotin is critical to the materials exhibiting an ability to inhibit cell growth.

Finally, it is found that some compounds preferentially inhibit MDA-MB-231growth compared to MCF-7 growth. The simple explanation is that the MCF-7 cells are complexing (or some other mode to render some of the applied drug less active) the test compound whereas the MDA-MB-231 cells do not allowing a greater amount of the drug to inhibit cell growth.

4. Summary

In general, all of the polymers containing biologically active and simple diols exhibit an ability to inhibit MCF-7 and MDA-MB-231 breast cancer cells. There does not appear to be any linkage to the organotin moiety that is markedly superior or inferior to inhibiting the MCF-7 and MDA-MB-231 breast cancer cell lines.

The concentration level for EC_{50} generally depends largely on the nature of the Lewis base and is only secondarily dependent on the nature of the organotin. Generally, EC_{50} values are lower and CI_{50} values are higher for the organotin polymers compared to the standard cisplatin. In fact, with respect to CI_{50} all of the compounds are superior (higher) to cisplatin. For EC_{50} and CI_{50} values, there does not appear to be a relationship between whether or not the Lewis base is biologically active. Thus, good EC_{50} and CI_{50} values are found for polymers for the hydroquinone group where the hydroquinone monomers do not exhibit inhibition of the MCF-7 and MDA-MB-231 cell lines and for the diethylstilbestrol polymers where diethylstilbestrol does inhibit MCF-7 and MDA-MB-231 cell growth.

With respect to EC_{50} values the thiamine and phentolamine polymers show low EC_{50} values but the monomers thiamine and phentolamine do not inhibit MCF-7 and MDA-MB-231 cell growth. By comparison, diethylstilbestrol itself inhibits cancer cell growth and the polymers derived from it also exhibit low EC_{50} values. Thus, it is the combination of the organotin and Lewis base that allows for inhibition of the MCF-7 and MDA-MB-231 cancer cell lines.

There is often little agreement between which compounds within a Lewis base group that exhibit the lowest EC_{50} and highest CI_{50} values. Unlike some other cancers, namely PC-3 prostate cancer, the steric nature of the Lewis base is not a major influence in the ability of the polymer to inhibit MCF-7 and MDA-MB-231 cell growth. This is consistent with the organotin polymers intersecting cancer growth at different locations for the PC-3 cancer cell line and the MCF-7 and MDA-MB-231cell lines.

There is a marked difference between some of the polymers and their ability to inhibit the growth of the MCF-7 and MDA-MB-231 cell lines. Polymers derived from the Lewis bases dienestrol, diethylstilbestrol, and a series of hydroquinone derivatives show superior ability to inhibit the MDA-MB-231 cell line in comparison to the MCF-7 estrogen positive cell line. It is believed that the MCF-7 cell line reacts with the polymers removing much of them so that their ability to inhibit cell growth is lessened.

Cell line testing has started, but this needs to continue. These further studies should solidify/modify/change many of the preliminary results cited here. Most of all, live animal testing must be carried out to see how valid the cell line results are with respect to inhibiting cancer growth within living subjects. This is being planned.

References

1. R. Siegel, CA Cancer J. Clin. **61**, 212 (2011)
2. P. D. Baade, D. R. Youlden, L. J. Krnjacki, Mol. Nutrition Food Res. **53**, 171 (2009)
3. NCI Breast Cancer, June 2014; NCI Breast Cancer Treatment, 2014
4. World Cancer Report 2014, World Health Origination, 2014 (ISBN 92-832-0429-8)
5. H. D. Soule, J. Vazguez, A. Long, S. Albert, M. Brennan, A human cell line from a pleural effusion derived from a breast carcinoma, J. National cancer Institute, 51, 1409-1416, 1973

6. R. Cailleau, R. Young, M. Olive, W. Reeves, Breast tumor cell lines from pleural effusions, J. National Cancer Institute, 53, 661-674, 1974.

7. M. Lacroix, G. Leclercq, Relevance of breast cancer cell lines as models for breast tumors: an update, Breast Cancer Res. Treat., 83, 249-289, 2004.
8. C. Carraher, *Macromolecules Containing Metal and Metal-Like Elements. Vol. 4. Group IVA Polymers*, Wiley, Hoboken, NJ, 2005, pp. 263-310.
9. C. Carraher, M. Roner, S. Shahi, Barot, Structural Considerations in Designing Organotin Polyethers to Arrest the Growth of Breast Cancer Cells *In Vitro*, Materials, 4, 801-815, 2011.
10. C. Carraher, M. Roner, M., Organotin Polyethers as Biomaterials, Materials, 2, 1558-1598, 2009.
11. C. Carraher, M. Roner, Organotin Polymers as Anticancer and Antiviral Agents, J. Organomet. Chem., 751, 67-82, 2014J. .
12. C. Carraher, Y. Naoshima, K. Nagao, Y. Mori, A. Zhao, G. Barot, A. Battin, in Inorganic and Organometallic Macromolecules, Springer, NY, pg 421
13. C. Carraher, M. Roner, K. Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin, N. Trang, M. Alhumiti, Control of Prostate Cancer Using Organotin Polymers, J. Organomet. Chem., in press.
14. C. Nebbia, M. Dacasto, L. Ceppa, M. Gennaro Soffietti, P. Spinelli, V. Bergo, P. Di Simplicio, Vet. Res. Commun. **21**, 117 (1997)
15. D. Siegmann-Louda, C. Carraher, Macromolecules Containing Metal and Metal-Like Elements. Vol. 3. Biomedical Applications,, Wiley, Hobokin, 2004.
16. Y. Luo, G.D. Prestwich, Cancer-targeted polymeric drugs, Curr. Cancer Drug Targets **2**, 209 (2002)
17. H. Maeda, Bioconjug. Chem. **21,** 797 (2010)

18.. J. Fang, H. Nakamura, H. Maeda, Adv. Drug Deliv. Rev. **63**, 136 (2011)
19. V. Torchilin, Adv. Drug Deliv. Rev. **63**, 131 (2011)
20. P. Goddard, I. Williamson, J. Brown, L.E. Hutchinson, J. Nicholls, K. Petrak, J. Bioact. Compat. Pol. **6**, 4 (1991)
21. P.C. Phillips, Neurologic Clinics, **9**, 383 (1991) 383
22. D.W. Siegmann-Louda, C.Carraher, F. Pflueger, J. Coleman, S. Harless, H. Luing, Biological Activity of Organotin Polymers Containing Cephalexin Against Human Ovary Adenocarcinoma Resistant Cell Lines, Polym. Mater. Sci. Eng. **82**, 83-84 (2000)
23. K. Abdellaoui, M. Boustta, H. Morjani, M. Manfait, M. Vert, J. Drug Target **5**, 193 (1998)
24. P.A. Vasey, S.B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A.H. Thomson, L.S. Murray, T.E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, J. Cassidy, Clin. Cancer Res. **5**, 83 (1999)
25. M.K. Chourasia, S.K. Jain, Drug Deliv. **11**, 129 (2004)
26. E. Fournier, C. Passirani, N. Colin, P. Breton, S. Sagodira, J.P. Benoit, Eur. J. Pharm. Biopharm. **57**, 189 (2004)
27. K. Ulbrich, V. Subr, Adv. Drug Deliv. Rev. **56** 1023 (2004)
28. C. Carraher, R. Chrichton, M. Roner, Synthesis of Organotin Polyamines from Reaction of 3-Amino-1,2,4-triazole and Organotin Dihalides Polym. Mater. Sci. Eng. , in press
29. C. Carraher, A. Campbell, M. Roner, Synthesis of Organotin Polyesters from Reaction of the Salt of D-Camphoric Acid and Organotin Dihalides, Polym. Mater. Sci. Eng, 111 (278), 1-3, 2014.
30. C. Carraher, M. Ayoub., M. Roner, A. Moric, N. Trang, Synthesis, Structural Characterization and Ability to Inhibit the Growth of Pancreatic Cancer by Organotin Polymers Containing Chelidonic Acid, J Chinese Advanced Materials Society, 1, 65-73, 2013.

31. C. Carraher, V. Suresh, M. Roner, Synthesis of Organotin Polyether Esters from Reaction of the Salt of Alpha-Cyano-4-Hydroxycinnamic Acid and Organotin Dihalides, Polym. Mater. Sci. Eng., 111 (279), 1-4, 2014

32. C. Carraher, N. Sookedo, J. Johnson, Roner, M., Synthesis of Organotin Polyethers Derived from the Anticoagulant Dicumarol, Polym. Mater. Sci. Eng., 111 (280), 1-3, 2014,
33. C. Carraher, M. Roner, Y. Ashida, G. Barot, Synthesis, Structural Characterization, and Anti-Cancer Evaluation of Group IVB-Metallocene Polyethers Containing the Synthetic Estrogen Diethylstilbestrol, J. Polym. Mater. 24, 357-369 (2007).
34. C. Carraher, M. Roner, K. Shahi, G. Barot, Synthesis and Initial Cell Line Results of Organotin Polyethers Containing Diethylstilbestrol, J. Inorg. Organometal. Polym., 18, 188-188 (2008)

35. C. Carraher, Y. Ashida, G. Barot, Synthesis of Organotin Polyether Containing the Sex Hormone Dienestrol, Poly. Mater. Sci. Eng., 101, 1405-1407, 2009.

36. C. Carraher, N. Truong, M. Roner, A. Moric-Johnson, N. Sookdeo, N. Trang, Synthesis of Organotin Poly(ether Esters) From Reaction With Glycyrrhetinic Acid and Their Preliminary Activity Against Various Cancer Cell Lines, Inorg. Chim. Acta, in press

37. C. Carraher, N. Truong, M. Roner, Synthesis of Organotin Polyether Esters from the Reaction of Organotin Dihalides with Glycyrrhetinic Acid, Polym. Mater. Sci. Eng., 105, 987-989, 2011.

38. C. Carraher, Z. Islam, M. Roner, Synthesis of Organotin Polyamines From the Interfacial Polycondensation Reaction of Histamine with Organotin Dihalides, Polym. Mater. Sci. Eng., 109 (172) 1-3, 2013.

39. C. Carraher, T. Arnold, M. Roner, Synthesis of Organotin Polyesters from the Reaction of Organotin Dihalides with Isomannide (D-Mannitol), Polym. Mater. Sci. Eng., 105, 990-992, 2011.

40. M. Roner, J. Williams-Sheffler, C. Carraher, Polym. Mater. Sci. Eng., Ability of Organotin Polyther Amines Derived from Phentolamine (Regitine) to Inhibit Ovarian, Colon, Lung, Prostrate, Pancreatic and Breast Cancer Cells, Poly. Mater. Sci. Eng., 102, 77-79, 2010.

41. C. Carraher, R. Lambert, M. Roner, Synthesis of Organotin Polyamine Esters Containing thiamine (Vitamine B1), Polym. Mater. Sci. Eng., 105, 983-986, 2011.

42. C. Carraher, N. Pham, M. Roner, Organotin Polyesters Containing thiodiglycolic Acid: Synthesis, Polym. Mater. Sci. Eng., 107, 345-347, 2012.

43. M. Hoch, Organotin in the Environment, Appl. Geochem. 16, 719-743 (2001).

44. K. Shahi, M. Roner, G. Barot, C. Carraher, Molecular Weight Dependency on the Ability of Organotin Polyethers Based on Hydroxyl-Terminated Poly(ethylene glycols) to Inhibit Cancer Cell Growth, Poly. Mater. Sci. Eng., 98, 390-392, 2008

45. G, Barot, C. Carraher, Molecular Weight Control of Organotin Polyethers Synthesized by Interfacial Polymerization, Poly. Mater. Sci. Eng., 98, 387-389, 2008

46. C. Carraher, G. Barot, S. Vetter, G. Nayak, M. Roner, Degradation of the Organotin Polyether Derived From Dibutyltin Dichloride and Hydroxyl-Capped Poly(ethylene Glycol) in Trypsin and Evaluation of Trypsin Activity Employing Light Scattering Photometry and Gel Electrophoresis, J. Chinese Advanced Materials Society, 1, 1-6(2013)

47. C. Carraher, J. L. Lee, Synthesis of Simple Polyethers Utilizing a New Technique, Polym. Mater. Sci. Eng., 90, 408-409 (2004).

48. C. Carraher, G. Scherubel, Production of Organometallic Polymers by the Interfacial Technique XXII. Synthesis of Poly(alkylene(arylene)oxystannanes) Using a New Modified System, Makromolekulare Chemie, 152, 61-66 (1972)

49. C. Carraher, G. Scherubel, Synthesis of Poly(tin Ethers) Employing Alkoxides, Makromolekulare Chemie, 160, 259-261 (1972)

50. C. Carraher, G. Scherubel, Production of Organometallic Polymers by the Interfacial Technique XX. Synthesis of Poly(alkyloxystannanes), J. Polymer Sci., 9(A1), 983-989, 1971.

51. D. Siegmann-Louda, C. Carraher, D. Nagy, D. Snedden, J. Rosa, Simple Organotin Polyethers as Potential Anti-Cancer Drugs, Polym. Mater. Sci. Eng., 89, 487-489 (2003).

52. G. Barot, D. Siegmann-Louda, E. Zevalias, C. Carraher, Inhibition of Balb 3T3 Cells by Selected Organotin Poly(ethylene glycol) ethers and the Product from 2-Butyne-1,4-diol, Polym. Mater. Sci. Eng., 94, 561-563 (2006).
53. G. Barot, K. Shahi, M. Roner, C. Carraher, Synthesis, Structural Characterization, and Ability to Inhibit Cancer Growth of a Series of Organotin Poly(ethylene glycols), J. Inorg. Organometal. Polym., 17, 595-603 (2007)
54. C. Carraher, G. Barot, K. Shahi, M. Roner, Influence of DMSO on the Inhibition of Various Cancer Cells by Water Soluble Organotin Polyethers, J. Chinese Adv. Maters., 1, 294-304 (2013).
55. C. Carraher, M. Roner, K. Shahi, A. Battin, G. Barot, T. Arnold, Organotin polymers as Chemotherapeutic Agents: Breast and Pancreatic Cancers, J. Polym. Mater., 31, 1-14, 2014.
56. C. Carraher, G. Barot, M. Roner, K. Shahi, C. Pellerito, T. Fiore, M. Scopelito, L. Pellerito, Synthesis, Structural Analysis, Fiber Formation and Preliminary Anticancer Characterization of the Organotin Polyether from Dibutyltin Dichloride and 2,5-Dimethyl-3-hexyne-2,5-diol, J. Polym. Mater., 29, 361-376, 2012.
57. C. Carraher, G. Barot, K. Shahi, M. Roner, Synthesis, Anomalous Fiber Formation, and Preliminary Anticancer Study of the Organotin Polyether Derived from 2-Butyne-1,4-diol, J. Polymer. Mat., 23, 423-436, 2006.
58. M. Roner, K. Shahi, G. Barot, A. Batton, C. Carraher, Preliminary Results for the Inhibition of Pancreatic Cancer Cells by Organotin Polymers, J. Inorg. Organomet. Polym., 19, 410-414 (2009)
59. G. Barot, M. Roner, Y. Naoshima, K. Nagao, K. Shahi, C. Carraher, Synthesis, Structural Characterization, and Preliminary Biological Characterization of Organotin Polyethers Derived from Hydroquinone and Substituted Hydroquinones, JIOPM, 19, 12-27 (2009).

60. K. Shahi, M. Roner, G. Barot, C. Carraher, Ability to Inhibit Various Cancer Cell Lines by Dibutyltin Polyethers Derived from 1,1- and 1,2-Diols, Poly. Mater. Sci. Eng., 99, 349-351, 2008
61. A. Batton, K. Shahi, M. Roner, C. Carraher, Synthesis, Structural Characterization, and Initial Evaluation as Anticancer Drugs of Dibutyltin Polyamines Derived from Various 4,6-Diaminopyrimidines, J. Inor. Organometal. Polym., 17, 631-639 (2007)

Tables & Figures (Coupled for easy association between the data and structure)

Table 1 Results for the product of various organotin dihalides with 3-amino-1,2,4-triazole 3-AT[28]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	3-AT	8.0×10^4	21000(2100)	17000(2000)	1.2	20000(2000)	1.1
Et_2SnCl_2	3-AT	4.2×10^5	21000(2100)	14000(1000)	1.5	20000(2000)	1.1
Bu_2SnCl_2	3-AT	3.8×10^4	26000(2500)	24000(2000)	1.1	22000(2000)	1.1
Oc_2SnCl_2	3-AT	1.4×10^5	25000(2400)	29000(3000)	0.86	30000(3000)	0.83
$Ph_2Sn\ Cl_2$	3-AT	2.1×10^5	9500(1000)	25000(3000)	0.36	24000(2000)	0.38

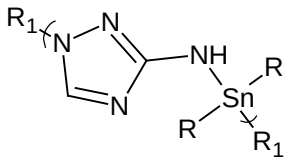


Fig. 1 Repeat unit for the product of organotin dihalides and 3-amino-1,2,4-triazole where R_1 represents chain extension.

Table 2 Results for the product of various organotin dihalides with camphoric acid[29]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	Camphoric acid	8.0×10^4	54(6)	67(8)	0.81	68970	0.79
Et_2SnCl_2	Camphoric acid	4.2×10^5	540(5)	43(4)	1.3	52(5)	1.0
Bu_2SnCl_2	Camphoric acid	3.8×10^4	55(5)	51(5)	1.1	48(5)	1.1
Oc_2SnCl_2	Camphoric acid	1.4×10^5	43(4)	47(4)	0.91	62(7)	0.69
$Ph_2Sn\ Cl_2$	Camphoric acid	2.1×10^5	54(5)	64(6)	0.84	53(6)	1.0
	Camphoric acid itself		1100(10)	1200(100)	0.92	1200(100)	0.92

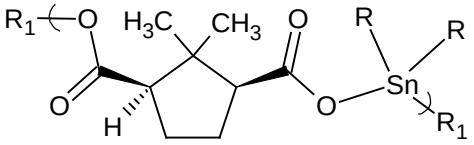


Fig. 2 Repeat unit for the product of camphoric acid and diorganotin dihalides where R represents simple chain extension.

Table 3 Results for the product of various organotin dihalides with chelidonic acid[30]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCA	CI_{50} MCF
Me_2SnCl_2	Chelidonic Acid	2.0×10^4	>60000	>60000		>60000	
Et_2SnCl_2	Chelidonic Acid	1.1×10^4	190(10)	19000(2200)	0.01	2900(17)	0.06
Bu_2SnCl_2	Chelidonic Acid	1.7×10^5	34(6)	2700(100)	0.012	240(6)	0.14
Cy_2SnBr_2	Chelidonic Acid	3.8×10^5	0.34(0.1)	34000(1000)	0.00001	14(5)	0.02
Oc_2SnCl_2	Chelidonic Acid	2.3×10^5	6000(220)			3300(9)	1.8
Ph_2SnCl_2	Chelidonic Acid	1.1×10^5	0.11(0.06)	2900(300)	0.00001	120(4)	0.001
	Chelidonic Acid		>60000	>60000		25000(260)	>1.3

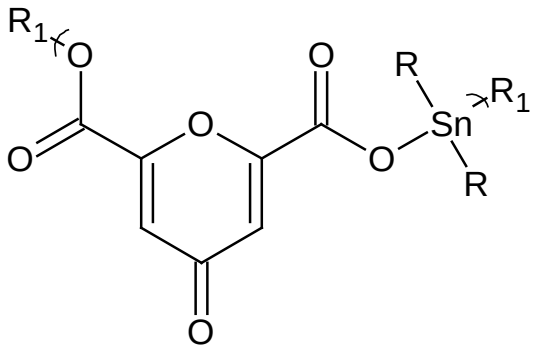


Fig. 3 Repeat unit for the product of organotin dihalides and chelidonic acid where R_1 represents chain extension.

Table 4 Results for the product of various organotin dihalides with cyano-4-hydroxycinnamic acid, CHCA[31]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	CHCA	8.0×10^4	35000(3500)	38000(400)	0.92	33000(3000)	1.1
Et_2SnCl_2	CHCA	4.2×10^5	17000(1500)	20000(2000)	0.85	19000(2000)	0.89
Bu_2SnCl_2	CHCA	3.8×10^4	26000(2600)	27000(3000)	0.98	29000(3000)	0.90
Oc_2SnCl_2	CHCA	1.4×10^5	20000(2000)	21000(2000)	0.98	23000(2000)	0.87
Ph_2SnCl_2	CHCA	2.1×10^5	16000(1500)	21000(2000)	0.76	20000(2000)	0.80

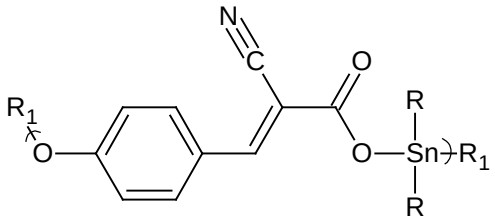


Fig. 4 Repeat unit for the product of organotin dihalides and cyano-4-hydrocinnamic acid where R_1 represents chain extension.

Table 5 Results for the product of various organotin dihalides with dicumarol[32]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	Dicumarol	7.3×10^4	10000(1100)	11000(1100)	0.91	14000(1300)	0.71
Et_2SnCl_2	Dicumarol	1.9×10^4	20000(2000)	22000(2000)	0.91	20000(2000)	1.0
Bu_2SnCl_2	Dicumarol	4.0×10^4	9900(1000)	11000(1100)	0.91	11000(1000)	0.91
Cy_2SnBr_2	Dicumarol	2.3×10^5	11000(1100)	12000(1200)	0.92	12000(1100)	0.92
$Oc_2Sn\ Cl_2$	Dicumarol	1.6×10^5	11000(1100)	11000(1100)	1.0	11000(1000)	1.0
$Ph_2Sn\ Cl_2$	Dicumarol	2.2×10^4	11000(1000)	13000(1400)	0.77	11000(1100)	0.91

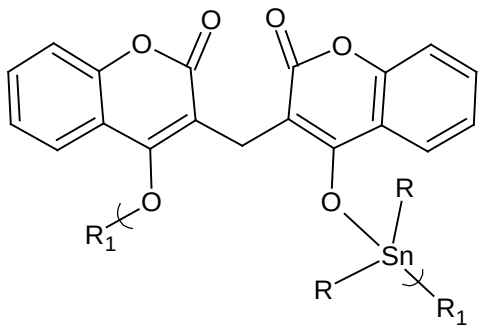


Fig. 5 Repeat unit for the product of organotin dihalides and dicumarol where R_1 represents chain extension.

Table 6 Results for the product of various organotin dihalides with diethylstilbestrol[33,34]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	Diethylstilbestrol	6.4×10^4	1600(500)	470(40)	**3.4**	150(50)	**2.5**
Et_2SnCl_2	Diethylstilbestrol	2.1×10^4	50(10)	160(10)	0.31	550(50)	0.9
Pr_2SnCl_2	Diethylstilbestrol	1.2×10^4	2300(500)	90(10)	**2.6**	660(50)	**3.5**
Bu_2SnCl_2	Diethylstilbestrol	1.1×10^4	2500(500)	50(10)	**50**	620(50)	**4.0**
Cy_2SnBr_2	Diethylstilbestrol	9.3×10^3	220(20)	210(20)	1.0	500(50)	**4.4**
Ph_2SnCl_2	Diethylstilbestrol	1.1×10^5	2300(200)	110(20)	**21**	650(50)	**3.5**
	Diethylstilbestrol		250(20)	50(10)	**5.0**	640(50)	0.39

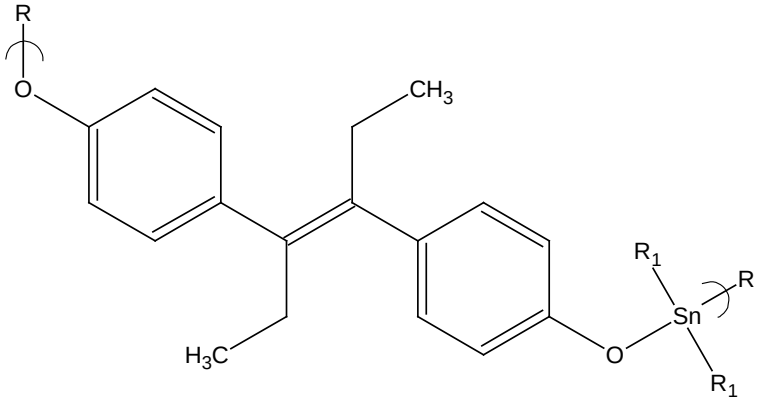


Fig. 6 Repeat unit for the product of organotin dihalides and diethylstilbestrol where R_1 represents chain extension.

Table 7 Results for the product of various organotin dihalides with dienestrol[35]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	Dienestrol	1.3×10^6	1500(500)	130(60)	**12**	760(60)	**2**
Et_2SnCl_2	Dienestrol	1.8×10^6	1400(500)	40(10)	**35**	810(60)	1.7
Pr_2SnCl_2	Dienestrol	2.6×10^6	310(200)	210(20)	1.6	690(50)	0.5
Bu_2SnCl_2	Dienestrol	1.3×10^6	60(10)	30(10)	2.0	760(50)	0.1
Cy_2SnBr_2	Dienestrol	1.4×10^6	260(200)	240(20)	1.1	700(50)	0.4
Ph_2SnCl_2	Dienestrol	2.4×10^6	190(20)	310(40)	0.6	700(50)	0.3
	Dienestrol		250(200)	110(20)	**2.2**	440(50)	0.6

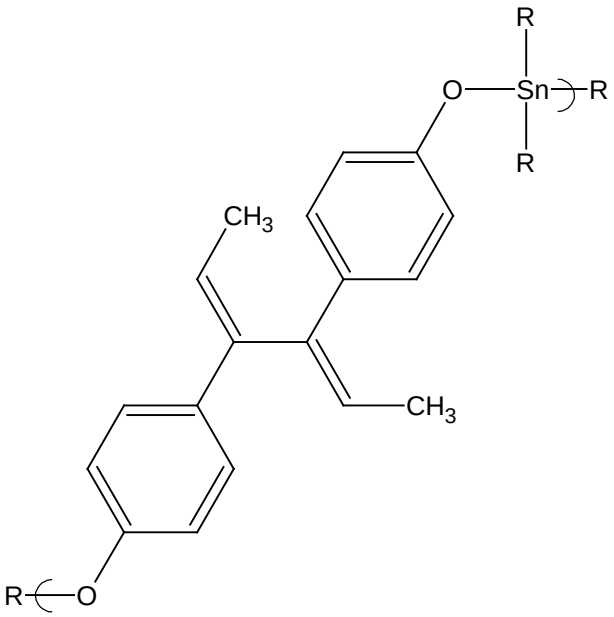


Fig. 7 Repeat unit for the product of organotin dihalides and dienestrol where R_1 represents chain extension.

Table 8 Results for the product of various organotin dihalides with glycyrrhetinic acid, GlyA[36,37]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	GlyA	7.3×10^5	1.1(0.1)	26(10)	0.04	17(10)	0.06
Et_2SnCl_2	GlyA	1.0×10^4	4.5(0.1)	3300(200)	0.001	2800(300)	0.001
Bu_2SnCl_2	GlyA	1.5×10^5	9.4(0.6)	310(40)	0.03	300(40)	0.03
Cy_2SnBr_2	GlyA	5.3×10^4	160(5)	9900(1100)	0.02	2300(300)	0.07
Ph_2SnCl_2	GlyA	2.0×10^4	1.0(0.1)	1600(200)	0.001	1400(200)	0.001

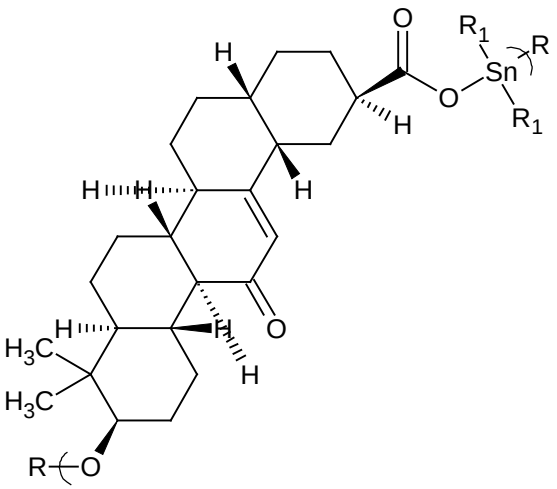


Fig. 8 Repeat unit for the product of organotin dihalides and glycyrrhetinic acid where R_1 represents chain extension.

Table 9 Results for the product of various organotin dihalides with histamine.[38]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Me_2SnCl_2	Histamine	6.2×10^4	17000(3000)	560(100)	**30**	2000(100)	**8.5**
Et_2SnCl_2	Histamine	2.0×10^5	16000(2500)	31(20)	**520**	46(20)	**350**
Bu_2SnCl_2	Histamine	1.4×10^4	12000(4400)	18(20)	**670**	33(20)	**360**
Cy_2SnBr_2	Histamine	2.4×10^5	30000(6700)	440(200)	**68**	360(100)	**83**
Oc_2SnCl_2	Histamine	9.2×10^5	31000(6500)	2100(1000)	**15**	32000(2200)	0.97
$Ph_2Sn\ Cl_2$	Histamine	1.1×10^5	12000(4500)	420(20)	**29**	190(100)	**63**

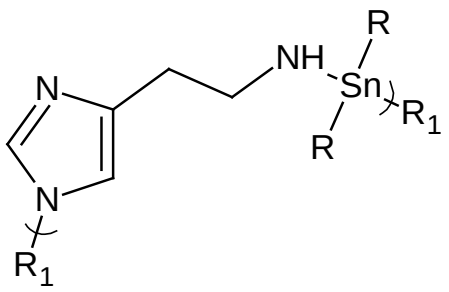


Fig. 9 Repeat unit for the product of organotin dihalides and histamine where R_1 represents chain extension.

Table 10 Results for the product of various organotin dihalides with isomannide[39]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Me_2SnCl_2	Isomannide	2.6×10^4	3.4(1)	1.5(1)	**2.3**	5.1(0.2)	0.67
Et_2SnCl_2	Isomannide	4.4×10^4	>60,000	>60,000		>60,000	
Bu_2SnCl_2	Isomannide	2.2×10^4	>60,000	>60,000		>60,000	
Cy_2SnBr_2	Isomannide	1.1×10^5	10(1)	9000(170)	0.001	4400(100)	0.002
Ph_2SnCl_2	Isomannide	5.7×10^6	>60,000	>60,000		>60,000	

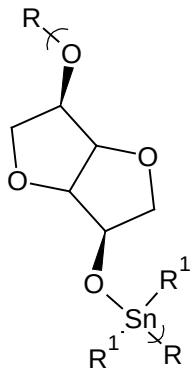


Fig. 10 Repeat unit for the product of organotin dihalides and isomannide where R_1 represents chain extension.

Table 11 Results for the product of various organotin dihalides with phentolamine (Regitine)[40]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Me_2SnCl_2	Phentolamine	1.4×10^5	250(100)	730(100)	0.34	700(100)	0.36
Et_2SnCl_2	Phentolamine	4.2×10^4	200(100)	100(10)	**2.0**	550(100)	0.36
Bu_2SnCl_2	Phentolamine	1.5×10^5	250(100)	580(100)	0.43	860(100)	0.29
Ph_2SnCl_2	Phentolamine	2.2×10^5	200(100)	450(100)	0.44	100(10)	**2.0**

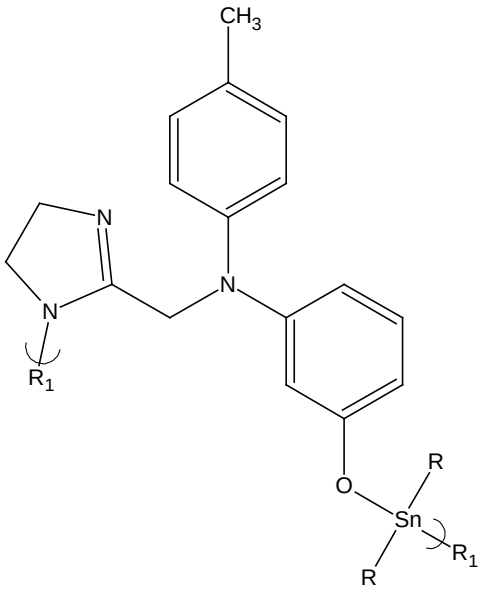


Fig. 11 Repeat unit for the product of organotin dihalides and phentolamine where R_1 represents chain extension.

Table 12 Results for the product of various organotin dihalides with thiamine[41]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	Thiamine	1.4×10^5	500(100)	500(100)	1	610(100)	0.82
Et_2SnCl_2	Thiamine	4.2×10^5	550(100)	620(100)	0.89	900(100)	0.61
Bu_2SnCl_2	Thiamine	1.5×10^5	450(100)	700(100)	0.64	750(100)	0.93
Ph_2SnCl_2	Thiamine	2.2×10^5	450(100)	400(100)	1.1	430(100)	1.1

Fig. 12 Repeat unit for the product of organotin dihalides and thiamine where R_1 represents chain extension.

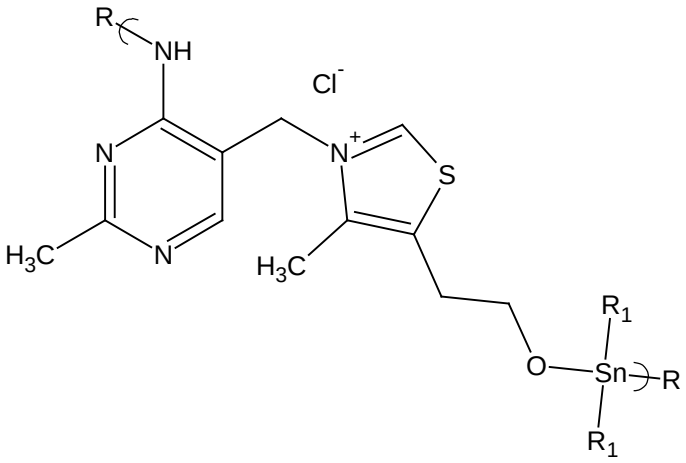


Fig. 12 Repeat unit for the product of organotin dihalides and thiamine where R represents chain extension.

Table 13 Results for the product of various organotin dihalides with thiodiglycolic acid[42]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Me_2Sn	Thiodiglycolic Acid	7.1×10^4	1600(120)	>60000		>32000	<0.5
Et_2Sn	Thiodiglycolic Acid	2.9×10^5	440(48)	1700(100)	0.26	4000(26)	0.11
Bu_2Sn	Thiodiglycolic Acid	1.3×10^5	12(6)	2.1(1)	**5.7**	54(6)	0.22
Cy_2Sn	Thiodiglycolic Acid	1.1×10^4	0.11(0.09)	1.7(1)	0.06	15(6)	0.01
Ph_2Sn	Thiodiglycolic Acid	5.0×10^5	3.3(2.4)	33(2)	0.1	140(10)	0.02

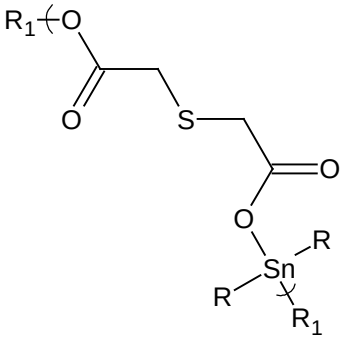


Fig. 13 Repeat unit for the product of organotin dihalides and thiodiglycolic acid where R_1 represents chain extension.

Table 14 Inhibition results for the organotin monomers

Lewis Acid	EC_{50} WI-38	EC_{50} MDA	CI_{50}MDA	EC_{50} MCF	CI_{50} MCF
Me_2SnCl_2	220(100)	440(100)	0.50	660(100)	0.33
Et_2SnCl_2	200(100)	640(100)	0.31	770(100)	0.26
Pr_2SnCl_2	250(100)	470(100)	0.63	450(100)	0.56
Bu_2SnCl_2	200(50)	1400(100)	0.17	700(100)	0.29

Ph_2SnCl_2	250(100)	760(100)	0.33	680(100)	0.37
Cy_2SnCl_2	200(100)	450(100)	0.44	590(100)	0.34
Oc_2SnCl_2	300(100)	650(100)	0.46	700(100)	0.43
Bz_2SnCl_2	200(100)	750(100)	0.27	600(100)	0.33
Cisplatin	50(40)	1000(100)	0.05	3000(280)	0.02

Table 15 Best EC_{50} and CI_{50} values for each Lewis base group.

Lewis Base	**Lowest EC_{50} MDA**	**Highest CI_{50} MDA**	**Lowest EC_{50} MCF**	**Highest CI_{50} MCF**
3-Amino-1,2,4-triazole	Et_2Sn 14000	Et_2Sn 1.5	Me_2Sn & Et_2Sn 2000	Me_2Sn, Et_2Sn& Bu_2Sn 1.1
Camphoric acid	Et_2Sn 43	Et_2Sn 1.3	Bu_2Sn 48	Bu_2Sn 1.1
Chelidonic acid	Bu_2Sn 2700	Et_2Sn 0.01	Cy_2Sn 14	Oc_2Sn 1.8
Cyano-4-hydroxycinnamic acid	Et_2Sn 20000	Bu_2Sn & Oc_2Sn 0.98	Et_2Sn 19000	Me_2Sn 1.1
Dicumarol	Me_2Sn, Bu_2Sn & Oc_2Sn 11000	Cy_2Sn 0.92	Bu_2Sn,Oc_2Sn &Ph_2Sn11000	Et_2Sn 1; Oc_2Sn 1.0
Diethylstilbestrol	Bu_2Sn 50	Bu_2Sn 50	Me_2Sn 150	Cy_2Sn 4.4
Dienestrol	Bu_2Sn 30	Et_2Sn 35	Pr_2Sn 690	Me_2Sn 2.0

Glycyrrhetinic acid	Me_2Sn 20	Me_2Sn 0.04	Me_2Sn 17	Cy_2Sn 0.07
Histamine	Bu_2Sn 18	Bu_2Sn 670	Me_2Sn 31	Bu_2Sn 360
Isomannide	Me_2Sn 1.5	Me_2Sn 2.3	Me_2Sn 5.1	Me_2Sn 0.67
Phentolamine	Et_2Sn 100	Et_2Sn 2.0	Ph_2Sn 100	Ph_2Sn 2.0
Thiamine	Me_2Sn 500	Ph_2Sn 1.1	Ph_2Sn 430	Ph_2Sn 1.1
Thiodiglycolic acid	Cy_2Sn 1.7	Bu_2Sn 5.7	Cy_2Sn 15	Bu_2Sn 0.22
Cisplatin	1000	0.05	3000	0.02

Table 16 Inhibition results as a function of chain length.[45]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Bu_2SnCl_2	1,5-Pentanediol	1.8×10^4	50(10)	90(40)	0.56	200(10)	0.25
Bu_2SnCl_2	1,5-Pentanediol	1.6×10^4	80(10)	2100(100)	0.04	290(30)	0.28
Bu_2SnCl_2	1,5-Pentanediol	1.3×10^4	870(20)	330(30)	0.26	320(30)	0.27
Bu_2SnCl_2	1,5-Pentanediol	1.06×10^4	230(30)	1900(200)	0.12	610(30)	0.38

Bu_2SnCl_2	1,3-Propanediol	3.0×10^4	50(10)	900(100)	0.06	1100(100)	0.05
Bu_2SnCl_2	1,3-Propanediol	2.45×10^4	170(30)	490(40)	0.34	350(30)	0.47
Bu_2SnCl_2	1,3-Propanediol	2.07×10^4	46(10)	490(40)	0.09	820(50)	0.06
Bu_2SnCl_2	1,3-Propanediol	1.74×10^4	50(10)	480(40)	0.09	110(10)	0.41

Table 17 Cell growth for dibutyltin polyethers.[53-58]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Bu_2SnCl_2	Ethylene Glycol	1.0×10^4	900(100)	300(23)	3.0	1000(100)	0.90
Bu_2SnCl_2	1,3-Propanediol	3.0×10^4	50(10)	900(100)	0.56	1100(100)	0.05
Bu_2SnCl_2	1,4-Butanediol		60(10)	220(20)	0.27	150(20)	0.4
Bu_2SnCl_2	1,5-Pentandeiol	1.8×10^4	50(10)	90(40)	0.56	200(10)	0.25
Bu_2SnCl_2	1,6-Hexanediol	2.1×10^5	50(10)	350(40)	0.14	220(20)	0.23
Bu_2SnCl_2	1,7-Heptanediol	1.2×10^4	40(10)	100(10)	0.40	200(10)	0.20
Bu_2SnCl_2	1,8-Octanediol	4.0×10^4	20(10)	90(10)	0.22	220(30)	0.09

Bu_2SnCl_2	Diethylene Glycol	1.1×10^5	1200(100)	1200(100)	1.0	1200(100)	1.0
Bu_2SnCl_2	Triethylene Glycol	1.7×10^5	1100(100)	1200(100)	0.92	1200(110)	0.92
Bu_2SnCl_2	Pentaethylene Glycol	4.7×10^5	50(10)	900(10)	0.06	1200(10)	0.04
Bu_2SnCl_2	PEG-400	7.6×10^4	280(30)	2400(220)	0.12	1400(100)	0.02
Bu_2SnCl_2	PEG-8,000	1.1×10^5	1000(100)	10000(930)	0.10	10000(960)	0.10
Bu_2SnCl_2	PEH-10,000	7.6×10^4	1000(100)	10000(970)	0.10	1000(1000)	0.10
Bu_2SnCl_2	2,5-Dimethyl-6-hexyne-2,5-diol	1.35×10^5	210(100)	220(10)	0.5	400(100)	0.1
Bu_2SnCl_2	2-Butyne-1,4-diol	1.2×10^5	3000(280)	1400(120)	**2.1**	1400(120)	**2.3**

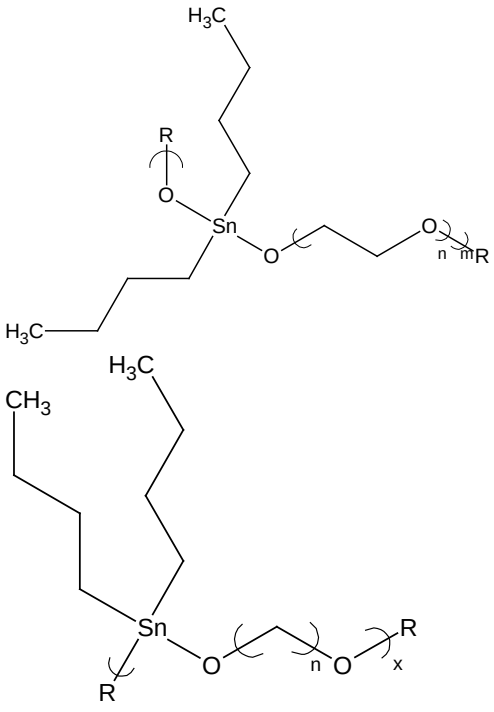


Fig. 14 Repeat unit for the polyethers derived from reaction of dibutyltin dichloride and various hydroxyl-terminated ethylene oxides (right) and methylene spacer diols (left) where R represents chain extension.

Table 18 Yield and molecular weight data for the dibutyltin polyethers derived from hydroquinone and the hydroquinone derivatives [59]

Lewis Acid	Lewis Base	Molecular Weight	EC_{50} WI-38	EC_{50} MDA	CI_{50} MDA	EC_{50} MCF	CI_{50} MCF
Bu_2SnCl_2	Methoxyhydroquinone	4.2×10^4	2000(300)	910(10)	**21**	1700(400)	1.2
Bu_2SnCl_2	Tert-Butylhydroquinone	3.3×10^4	1800(500)	230(100)	**7.6**	2400(500)	0.72

Bu_2SnCl_2	2,5-Di-tert-Butylhydroquinone	2.0×10^4	2200(500)	220(100)	**9.8**	1800(500)	1.2
Bu_2SnCl_2	Methylhydroquinone	2.9×10^4	2600(500)	36(10)	**71**	1700(400)	1.5
Bu_2SnCl_2	Phenylhydroquinone	3.3×10^4	210(30)	0110(10)	1.9	1700(400)	0.12
Bu_2SnCl_2	Hydroquinone	2.2×10^4	2000(500)	45(10)	**43**	1700(500)	1.1
Bu_2SnCl_2	2,3-Dicyanohydroquinone	8.3×10^4	2400(500)	220(90)	**11**	1900(500)	1.2
Bu_2SnCl_2	Bromphydroquinone	2.6×10^4	250(100)	85(10)	**2.9**	2700(400)	0.090
Bu_2SnCl_2	Chlorohydroquinone	5.9×10^4	10000(0300)	86(10)	**21**	1700(400)	1.1
Bu_2SnCl_2	2,5-Dichlorohydroquinone	2.5×10^4	1900(300)	380(90)	**4.9**	2600(500)	0.72
Bu_2SnCl_2	Tetrachlorohydroquinone	1.9×10^5	2200(500)	120(10)	**19**	3900(500)	0.57
Bu_2SnCl_2	2,5-Dihydroxybenzaldehyde	6.8×10^4	2000(500)	130(10)	**15**	2400(500)	0.84

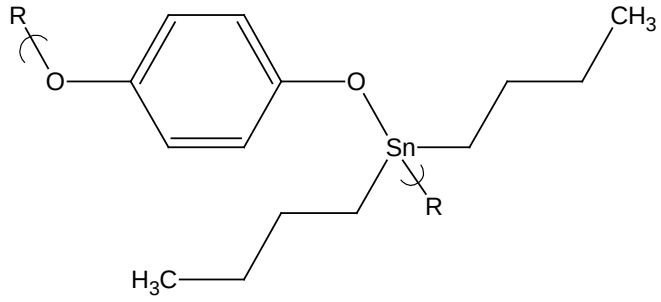


Fig. 15 Repeat unit for the product of organotin dihalides and hydroquinone where R represents chain extension.

Table 19 Product biological activities for organotin polyethers derived from 1,1- and 1,2-diols [60]

Lewis Acid	**Lewis Base**	**Molecular Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Bu_2SnCl_2	2,3-Butanediol	5.6×10^4	1100(300)	290(80)	**4.8**	420(100)	**2.6**

Bu_2SnCl_2	Ethylene Glycol	1.0 x 10^4	900(100)	300(23)	**3.0**	1000(100)	0.90
Bu_2SnCl_2	3-Chloro-1,2-propanediol	4.5 x 10^4	1900(400)	410(90)	**9.5**	1560(300)	1.2
Bu_2SnCl_2	Neopentyle Glycol	8.1 x 10^4	150(100)	110(10)	**3.0**	510(100)	0.29

Table 20 Inhibition results for dibutyltin dichloride polyamines derived from 4,6-diaminopyrimidine and substituted 4,6-diaminopyrimidines [61]

Lewis Acid	**Diamine**	**Mol Weight**	**EC_{50} WI-38**	**EC_{50} MDA**	**CI_{50} MDA**	**EC_{50} MCF**	**CI_{50} MCF**
Bu_2SnCl_2	4,6-Diamino-pyrimidine	3.7x10^6	1100(100)	1400(120)	0.85	1100(100)	1.0
Bu_2SnCl_2	4,6-Diamino-5-nitro-pyrimidine	1.3x10^5	1100(100)	1300(100)	0.85	1100(100)	1.0
Bu_2SnCl_2	4,6-Diamino-2-methyl-mercaptopyrimidine	1.8x10^5	1400(100)	1300(100)	1.0	1000(100)	1.4
Bu_2SnCl_2	4,6-Diamino-2-methyl-5-nitrosopyrimidine	5.5x10^5	1400(100)	1400(120)	0.93	1000(100)	1.4
Bu_2SnCl_2	4,6-Diamino-2-mercaptopyrimidine	1.8x10^6	250(10)	1500(130)	1.9	300(20)	0.7
Bu_2SnCl_2	4,6-Diamino-2-chloropyrimidine	1.4x10^5	40(30)	110(20)	0.04	300(40)	0.13

Bu_2SnCl_2	4,6-Diamino-2-hydroxylpyrimidine	$2.1x10^5$	50(10)	1100(40)	0.14	900(140)	0.06
Bu_2SnCl_2	4,6-Diamino-1-nitroso-2-hydroxylpyrimidine	$2.8x10^5$	80(10)	350(10)	0.80	750(80)	0.11
Bu_2SnCl_2	4,6-Diamino-5-(4-chloro- phenyl)-6-ethylpyrimidine	$3.5x10^4$	250(30)	100(10)	**2.5**	600(40)	0.42

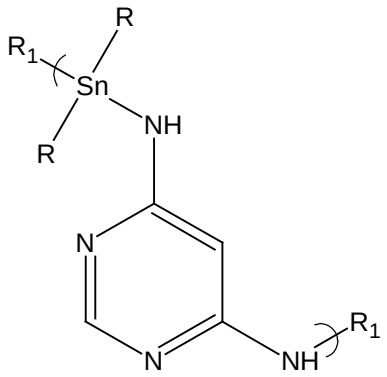


Fig. 16 Repeat unit for the product of organotin dihalides and 4,6-diaminopyrimidine where R_1 represents chain extension.

CHAPTER 6

SYNTHESIS OF ORGANOTIN POLYAMINE ETHERS CONTAINING THIAMINE (Vitamin B_1) AND PRELIMINARY ABILITY TO INHIBIT SELECT CANCER CELL LINES[4]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 9 and 10) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Roner, M.R.; Lambert, R. E.**;** Arroyo, L. and **Miller, L**

Carraher Jr.,C.E.; Roner, M.R.; Lambert, R. E.**;** Arroyo, L. and **Miller, L. 2015**. Synthesis of Organotin Polyamine Ethers Containing Thiamine (Vitamin B_1) and Preliminary Ability to Inhibit Select Cancer Cell Lines. J. Inorg. Organomet. Polym. 25:1414-1424. DOI 10.1007/s10904-015-0254-x

[4] Used with permission of Springer, 2017

Synthesis of Organotin Polyamine Ethers Containing Thiamine (Vitamin B_1) and Preliminary Ability to Inhibit Select Cancer Cell Lines

Charles E. Carraher, Jr.,[a] Michael R. Roner ,[b] Raven E. Lambert,[a] Luis Arroyo, [a] and Lindsey C. Miller[b]

a.Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 (carraher@fau.edu)

b.University of Texas at Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Abstract Organotin polyamine ethers containing thiamine (Vitamin B_1) were synthesized employing the interfacial polymerization of thiamine and organotin dichlorides. Reaction is rapid occurring in less than 15 seconds in moderate to poor yield with chain length ranging from 86 to 860 units. Infrared spectroscopy shows new bands characteristic of the formation of the Sn-N and Sn-O linkages. Proton NMR shows the presence of both the organotin and thiamine moieties. MALDI MS results shows ion fragment clusters to three units with ion abundance results consistent with the presence of tin atoms within the ion fragment clusters. The polymers all exhibit inhibition of the cancer cell lines including two breast, two pancreatic, prostate, and colon cancer cell lines at levels better than those for the standard cisplatin, one of the most widely employed anticancer drugs.

Keywords Thiamine-containing polymers, organotin polymers, MALDI MS, interfacial polycondensation, pancreatic cancer, breast cancer

1 Introduction

The biological activity of organotin compounds has been known for over 100 years and for more than 80 years organotin compounds have been known to inhibit cancer cell growth [1-3]. Further, more organotin compounds have undergone testing as potential anticancer agents than any other single group of compounds [1-4]. More organotin compounds are available commercially than any other metal-containing organometallic [1-3].

Much of our recent effort focused on the synthesis and biological characterization of organotin polymers. Many of them have arrested the growth of a number of cancer cell lines including those associated with lung, bone, colon, breast, ovarian, and prostate cancer and most recently, pancreatic cancer cell lines. These products generally exhibit good inhibition of cell growth equal to and greater than cisplatin, one of the most widely employed chemotherapeutic drugs. This has been recently reviewed [4].

The mechanism(s) of action of tin-containing compounds is not well-understood and has been recently summarized as has the advantages of employing polymeric drugs [2-18]. The locations of activity are numerous and these multiple sites may be advantageous in fighting cancer since they may allow curtailment of cancer growth through several mechanisms [4].

Much of our present syntheses have been driven by the idea that coupling the biological activity of the organotin moiety with that of Lewis bases that also exhibit biological activity may produce products that exhibit some synergetic activity giving the desired anticancer and antiviral activities [4]. For the present study the biologically active Lewis base is thiamine, TH or Th

Thiamine is a water soluble vitamin B complex also known as vitamin B_1 [19]. Its phosphate derivatives are important in many cellular processes. For instance, thiamine diphosphate, ThDP or TDP, is a coenzyme in the catabolism of amino acids and sugars [19]. A lack of B_1 leads to beriberi that affects the peripheral nervous system and the cardiovascular system. Thiamine is unstable to heat so that its incorporation in polymers must be carried out at low temperatures. The interfacial system is ideal in that the polymerization is carried out at room temperature [20-23].

Thiamine is released by the action of phosphatase and pyrophosphatase in the upper small intestine [24-27]. Most thiamine in serum is protein bound, mainly to albumin. In blood most thiamine is contained in erythrocytes. Blood cells, and other tissues, uptake thiamine through

active transport and passive diffusion [24-27]. Most intercellular thiamine is phosphorylated and bound to proteins. The highest concentration of thiamine is in skeletal muscle, brain, heart, liver, and kidneys.

Thiamine is the transport form of the vitamin but the active compounds are the phosphorylated forms [24-27]. Thiamine is capable of crossing cell membranes. Thus, it may assist the transfer of organotin-containing moieties into cells.

The presence of the amine salt allows association with other anions that may allow for better solubility of the polymer including eventually water solubility [4].

Reaction with divalent metal ions, M^{+2}, is required for all thiamine-diphate-dependent enzymes to be active [28]. The complex that is formed from the thiamine derivative and the metal ions is the true cofactor [29]. Thus, effort has been aimed at creating and studying various metal-complexes of thiamine and thiamine derivatives.

Synthesis of organotin-thiamine compounds has been achieved. For instance, the complex $[Sn(CH_3)_2HTDP)(H_2O]Cl$ has been synthesized from reaction of dimethyltin dichloride and thiamine diphosphate hydrochloride, H_3TPDCl, in water. Here, reaction occurred between the phosphate group and organotin forming P-O-Sn bonds rather than reaction with the thiamine. This is expected since the phosphate salt is a powerful nucleophile and should react at the electron-poor tin atom [30]. While hydrolysis of the dimethyltin dichloride forms hydroxo and/or oxohydroxo Sn (IV) derivatives with or without the chloride anions [31], the pH and concentration conditions employed in the previous study were such as to minimize this hydrolysis [30].

Fiore, et al., [32] obtained a different product from refluxing a methanolic suspension of $H_2TDP{\cdot}4H_2O$. Here products of the general form $R_2Sn(HTPP)_2{\cdot}nH_2O$ were formed. Thus, reaction

conditions are important in what the end product will be. We employed the simple Lewis acid-base reaction between thiamine and diorganotin dihalides to produce our products.

Several thiazolium-bound polymers have been synthesized for the general purpose of employing the bound-polymer matrix as a control release or actual reaction surface. Berg, Challa, and Pandit have synthesized such products binding them to linear and macroporous chloromethylated polystyrenes [33,34].

Related to this are the efforts to form vinyl polymers containing thiamine. Thus, poly(N-methacryloylcystein is mixed with thiamine disulfide to incorporate the thiamine moiety into the polymers. Other similar polymers have similarly been employed including poly(N-acryloylcysteine), poly(mercaptoethylene), poly(p-mercaptomethyl-styrene), poly(p-mercaptostyrene), and the copolymer of styrene and N-acryloylcysteine [35]. In another patient, poly(S-methacryloyl-O-benzoylthiamine was prepared from the polymerization of S-methacryloyl-O-benzoylthiamine [36]. Other similar polymers have also been reported [36].

Here we report the synthesis of polymers derived from the reaction of various organotin dihalides and thiamine (Fig. 1).

Fig. 1

2 Experimental

2.1 Synthesis

Thiamine (59-43-8) hydrochloride, dibutyltin dichloride (683-18-1), diphenyltin dichloride (1135-99-5), and dimethyltin dichloride (2767-47-7) were obtained from Aldrich Chemical Company. Diethyltin dichloride (866--55-7) was obtained from Peninsular. Reactions were carried out using

the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the thiamine (0.00300 mol) and sodium hydroxide (0.0090 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 ^{o}C). Stirring was begun and a hexane solution (30 ml) containing the organotin dihalide (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 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.

2.2 Physical Characterization

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 400 to 2,000. Fifty to two hundred shots were typically taken for each spectrum. Results employing alpha-cyano-4-hydroxycinnamic acid are included in the present paper. The solid product along with solid matrix were mixed together employing copper spheres giving a fine powder that was employed to obtain the spectra.

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the samples dissolved in 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 in d6 DMSO were obtained employing Varian Inova 400 MHz and Varian 500 MHz spectrometers.

2.3 Biological Characterization

Each of the cell lines listed in Table 1 were obtained from NCI and maintained in RPMI-1640 supplemented with 10% fetal bovine serum at 37^{o}C in a 5 % carbon dioxide atmosphere.

For testing of the compounds, cells were harvested, counted, and plated into 96-well plates at 1x10^{4} cells per well in RPMI-1640 supplemented with 10% fetal bovine serum, and incubated for 24 hours at 37^{o}C in a 5 % carbon dioxide atmosphere. A stock solution of the compound was

prepared in DMSO at a known concentration. On day two the cell media was removed and replaced with RPMI-1640 supplemented with 10% fetal bovine serum and the indicated drug concentrations. Forty-eight hours later the cells were assayed for proliferation using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay by Promega Corporation. Assays are performed by adding a small amount of the CellTiter 96 Aqueous One Solution Reagent directly to culture wells, incubating for 1–4 hours and then recording absorbance at 490nm with a 96-well plate reader. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture.

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

3 Results and Discussion

3.1 Yield and Chain Length

The reaction is general occurring for all of the organotin dichlorides. Percentage yields were poor (for the dibutyltin) to moderate (Table 1).

Table 1.

The products are moderate to high polymers with degrees of polymerization ranging from 86 to 860. There appears to be no relationship between yield and chain length. The lowest yield is for the dibutyltin product yet it has the highest chain length.

3.2 MALDI MS Results

Matrix-assisted desorption/ionization was independently introduced in 1981 by Barber and Liu and coworkers [37,38]. The addition of the laser as the energy source was introduced by Tanaka, Hillenkamp and coworkers in 1988 [39,40]. This combination of concepts allowed the creation of matrix-assisted laser/ desorption mass spectroscopy, MALDI MS. While MALDI MS was developed for the analysis of nonvolative samples with special use in the identification of polymers its potential for the analysis of a wide range of soluble and insoluble materials has not been fulfilled. This is largely due to the inability of many materials to be soluble in somewhat volatile liquids to a decent extent. This has largely eliminated most synthetic polymers from being suitably analyzed employing MALDI MS.

For about a decade we, and others, have been employing MALDI MS for the identification of a number of non-volatile metal and non-metal containing polymers [4]. The technique employed by us is not straight forward MALDI MS but it is applicable to soluble and insoluble products so has wide potential for application. Since this new technique focuses on the fragments that are created in the MALDI MS process, the approach is sometimes referred to as Fragmentation Matrix-Assisted Laser Desorption/ Ionization mass spectrometry or simply F MALDI MS because it is the fragments that are emphasized in the study. The technique should be applicable to any solid when the proper operating conditions are employed. There are some complications employing this technique to organotin because of rearrangements and the particular sensitivity of organotin moieties to laser radiation. The technique has been recently reviewed [41-43].

Interpretation of the MALDI MS for the current samples is not as straight forward as for most of the other compounds we have worked with because of the presence of the counter anion. We

believe this counter anion is the chloride ion because the original salt is the chloride and no other anion-containing unit is added. The question is whether it is the chloride-containing thiamine units that will be captured in the MS ion fragment clusters (Fig 2, left) or is it the chloride-free anion that is captured (Fig 2, right) or some combination.

Fig 2

We believe it is the chloride-free thiamine unit that is most prevalent in the ion fragment clusters observed for the various polymers. This is consistent with the type of MS employed- that is a negative ion system. Thus, positively charged species are favored to be detected. The polymer chains obviously contain the counter anion. The following description of the ion fragment clusters obtained from chain scission of the polymers is consistent with this.

Additionally, as reported elsewhere, there is the tendency for matrix-organotin ion fragment clusters to be formed [41-43]. These are omitted in the following tables.

Two general MALDI MS modes were employed. These are the reflective and linear mode. The reflective mode has a longer focal length than the linear mode. Results for the reflective mode allow the 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.

Results for the reflective mode for the product of dibutyltin dichloride and thiamine are given in Figure 3 and the major ion fragment clusters described in Table 2. Several abbreviations are employed in describing the tentative ion fragment clusters as follows: U = one repeat unit, 2U =

two repeat units. Sodium is a typical contaminant. All ion fragment masses are given in daltons (m/e=1).

Fig 3

Table 2

Ion fragment clusters to two and a half are found. There is loss of butyl groups from the organotin moiety. As already noted, this is common for organotin compounds since the tin-organic moieties are not stable to the radiation of the laser employed in the MALDI MS [41-43].

Isotopic abundance matches are employed to support the presence of tin atoms in the isotopic fragment clusters. Tin contains ten isotopes of which seven are considered significant. At higher masses, isotope matches are difficult because of the low intensities of generated ion fragments. At lower masses such isotope matches are possible. Table 3 contains the isotopic abundance match for the ion fragment cluster centered about 633 assigned as containing two tin atoms. The match is reasonable and consistent with this cluster containing two tin atoms.

Table 3.

For the product from diphenyltin dichloride and thiamine ion fragments are found to 930 using the reflective mode but when employing the linear mode ion fragments are found to nearly 2000. Table 4 contains the major ion fragment clusters for the linear mode. Ion fragments to three and a half units are found. For the reflective mode ion fragment clusters to 754 are found and include ion fragment clusters at 588, 677 and 754 found in the linear mode.

Table 4

Table 5

Table 5 contains two matches for ion fragment clusters containing two tin atoms. The abundance matches are consistent with the presence of two tin atoms within the particular ion fragment clusters.

Table 6 contains the major ion fragments found for the diethyltin product employing the linear mode. Abbreviations are also employed to describe the possible ion fragment clusters as follows: U = unit, 2U = two units; Th = thiamine moiety minus two hydrogen atoms.

Table 6

Ion fragment clusters to three units are found.

In summary, all of the MALDI MS ion fragment clusters containing tin have the typical isotopic fingerprint. Polymer chain cleavage occurs at the heteroatoms, as usual, as well as at the ethylene oxide-ring location. The "softness" of MALDI MS is shown by the lack of breakage of the thiamine ring. Results are consistent with the proposed repeat unit structure.

3.3 Infrared Spectrum Results

Infrared spectrums were obtained for the monomers and products. All bands are given in wave numbers, 1/cm. Table 7 contains results of this spectroscopy emphasizing the bands associated with the organotin moiety. Results for the dibutyltin product derived from the dibutyltin moiety are given in column 4.

Table 7

The Sn-O-C stretch is present at 1001 consistent with the formation of the Sn-O linkage to the thiamine. The band at 1152 is assigned to the Sn-N also consistent with the linkage to the thiamine. Bands at 1651 and 1601 are assigned to the C=C and C=N stretching in the pyrimidine ring for thiamine. The NH stretching band is present at about 3370 but the NH_2 band at about 3280 is missing, as expected. The band about 3490 assigned to the O-H stretching is also missing as expected.

Results for the product from diphenyltin dichloride are given in Table 7, column 6. The Sn-O-C stretch is present at 1022 and the Sn-N stretch is present at 1150, both consistent with the

formation of the linkages to the thiamine. Along with the C-H stretching vibrations from the tin-phenyl there are also C-H stretching vibrations from the thiamine occurring at about 3016 and 3010 associated with the aromatic rings of the thiamine and at 2992 associated with the aliphatic C-H stretching on thiamine. Bands at 1645 and 1598 are assigned to the C=C and C=N stretching in the pyrimidine ring from thiamine. The NH is present at 3587 but the NH_2 band at about 3280 is missing, as expected. Also missing is the O-H stretching band at about 3490 as expected.

Thus, the infrared vibrational spectral results are consistent with the proposed repeat unit structure.

3.4 Proton NMR

Proton NMR was taken for the products and is consistent with the product containing units from both of reactants. For the dibutyltin product, the methyl protons associated with the dibutyltin moiety are found at about 0.90 (dibutyltin 0.87)(all bands are given in ppm) (Fig. 4). The methylene protons are found at 1.2 to 1.3 and 1.6 (dibutyltin methylenes 1.2-1.6). Structural locations for the thiamine protons are given in Figure 4. The C2 proton is found at 9.45 (for thiamine itself 9.6); the C$^{'}$6 proton is found at 7.86 (thiamine 7.86); the C5$^{'}$ methylene associated protons are found at 5.4 (thiamine 5.5); the O-CH_2 protons are found at 4.1 (thiamine 4.07); C5 methylene associated protons are found at 3.2 (thiamine 3.21); C4 methyl protons are found at 2.55 (thiamine 2.6); and the C2$^{'}$ methyl protons are found at 2.5 (thiamine 2.5).

Fig 4

Fig 5

In summary infrared, NMR and mass spectra data is consistent with the proposed structure. What is not assigned is the precise arrangement about the tin. Thiamine is not symmetrical; three arrangements are possible about the tin. These are shown below.

R-O-Sn-O-R R-O-Sn-N-R R-N-Sn-N-R

Because the solubility of products is so low in d6 DMSO, we are not able to identify which of these arrangements predominate.

3.5 Cell inhibition

Much of our recent activity has focused on the synthesis and preliminary cancer cell line testing of various organotin-containing polymers. In general, as the organotin moiety is varied for a particular Lewis base, the polymer containing the dibutyltin moiety shows the greatest ability to curtail cancer cell line growth followed by those containing the diphenyltin moiety [4].

Table 8 contains the cell lines employed in the current study.

Table 8

While different measures have been employed in the evaluation of cell line results the most two widely employed are employed in the present study. The initial measure involves the concentration, dose, needed to reduce the growth of the particular cell line. Here the term effective concentration, EC is employed. 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}. The second measure is generally referred to as the Chemotherapeutic Index, CI_{50} which is a measure of the amount needed to inhibit 50% cell growth, GI_{50}, for a standard cell line divided by the amount needed to inhibit 50% cell growth for one of the cancer cell lines. Different researchers generally emphasize one of these measures over the other with neither measure universally accepted. Thus, results from both of these measures are presented. Cisplatin, one of the most widely used anticancer drugs, is used as the standard.

Table 9 contains the EC_{50} values for the monomers, polymers, and cisplatin.

Table 9

There are a number of observations derived from the data presented in Table 9. First, the polymers have a much greater toxicity towards the cancer cell lines compared with TH, which can be considered nontoxic over the studied range. Second, the polymers generally have a greater toxicity than cisplatin towards the tested cell lines showing EC_{50} values less than cisplatin. Third, the polymers and organotin monomers are both toxic towards all of the tested cell lines. Also, in general the toxic level of the polymers and organotin monomers are similar. If the toxicity were based only on the amount of

organotin moiety, then polymers exhibit a greater toxicity towards the cancer cell lines based on the amount of organotin moiety present.

Several observations with respect to individual cell lines results are appropriate. We will focus on the results for the polymers. 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 polymers it was found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure [4,44,45]. In these studies polymers containing a Lewis base that possesses the O-Phenylene moiety, such as diethylstilbestrol [45] and hydroquinone and hydroquinone derivatives [46], exhibits a relatively 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 TH in the present study, that do not contain this structural moiety showed little difference between the ability to inhibit the two cell lines [4]. Here, there is little difference between the ability to inhibit the two breast cancer cell lines with the various organotin polymers consistent with the thiamine not containing the O-phenylene moiety.

One of our recent focuses regards pancreatic cancer. Pancreatic cancer afflicts close to 32,000 individuals each year in the United States and 168,000 worldwide, and nearly all patients die from the ravages of their disease within 3 to 6 months after detection. 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). Treatment of pancreatic cancer is rarely successful as this disease typically metastasizes prior to detection. There is no chemotherapy for metastasized pancreatic cancer. We recently described the ability of a number of organotin polymers to inhibit pancreatic cancer [4,47-

50]. Because pancreatic cancer does not have a generally accepted "cure" one of our current focuses is on the synthesis of materials that might be successful in combating pancreatic cancer. 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. Both are human cell lines and are widely employed in testing for inhibition of pancreatic cancer. For the current study, all polymers show decent inhibition of both cell lines.

The PC-3 results are of interest because this particular prostate cell line is viewed as the most resistant of the prostate cancer cell lines [51]. Again, all of the polymers exhibit decent inhibition of the PC-3 cell line with all of them exhibiting EC_{50} values less than cisplatin and generally less than that shown for the organotin monomer.

Third, there is not a clear overall general trend with respect to the EC_{50} values so that the usual trend of the dibutyltin and diphenyltin containing polymers offering greater activity compared with other organotin-containing polymers is not present. Even so, the dibutyltin/TH polymer does offer decent inhibition of all of the tested cancer cell lines. This is important for several reasons [1,4]. First, it is the least expensive of the organotin halides and available in the ton and greater quantities. Second, it is the least toxic to humans of the lower alkyltins. Third, commercially it is the most widely employed organotin moiety [1]. Finally, the dibutyltin moiety is known to degrade in nature to the environmentally less toxic tin oxide [1].

In summary, the EC_{50} values are consistent with the combination of the organotin and thiamine moieties having a positive effect on the ability to inhibit cancer cell growth.

The second measure is the 50% chemotherapeutic index, CI_{50}. As noted before, the chemotherapeutic index is the concentration of the compound that inhibits the growth of the healthy cell by 50% divided by the concentration of the compound that inhibits the growth of the cancer cell

by 50%. Larger values are desired since they indicate that a larger concentration is required to inhibit the healthy cells in comparison to the cancer cells or stated in another way, larger values indicate some preference by the test samples for inhibiting the cancer cells in preference to the normal cells or standard cell line. In general, CI_{50} values larger than 2 are considered significant. CI_{50} values are given in Table 10.

Table 10

While the CI_{50} values for the organotin polymers and monomers are generally greater than for cisplatin, they are less than two consistent with the organotin polymers exhibiting little preference for arresting the cell growth of cancer cell lines.

A second study is superimposed onto the current study. This second study involves comparing results from the two most frequently used cell line standards involved in the evaluation of the effectiveness of compounds. These two cell lines are the NIH/3T3 and WI-38 cell lines.

NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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. While NIH/3T3 cells are often favored because of ease of handling aided by an infinite life span, results from WI-38 cells are given greater importance when there is a difference [52].

To see if the more easily handled NIH/3T3 cells can be used in place of the WI-38 cell one can simply observe the ratio of EC_{50} for the WI-38 values divided by the EC_{50} for the NIH/3T3. If there is no difference the ratio should be about 1. In the present case, the values range from 0.75 to 1.7 so there is not a significant difference. Thus, in the current study, little difference is found employing either standard. In other studies we have found mixed results where the use of 3T3 cells as the standard included samples that might otherwise be eliminated [4].

In summary, all of the polymers show ability to inhibit all of the cancer cell lines including the pancreatic cancer cell lines based on EC_{50}. Compared to cisplatin, the standard, they also exhibit lower EC_{50} values. Based on EC_{50} values, this group of polymers exhibits decent ability to inhibit all of the tested cancer cell lines.

4. Summary

Organotin polyamine ethers containing thiamine (Vitamin B_1) were synthesized employing the interfacial polymerization of thiamine and organotin dichlorides. The products are formed rapidly, less than 15 seconds, in poor to moderate yield. The products range from being short to long polymers. Since the reaction employs the interfacial reaction system that is used industrially to form aramids and polycarbonates, and the reactants are commercially available, synthesis of the products can be readily achieved in gram to ton quantity. Infrared spectroscopy shows new bands characteristic of the formation of the Sn-N and Sn-O linkages and the absence of OH and NH_2 both consistent with the formation of the desired polymer. Proton NMR shows the presence of both the organotin and thiamine moieties. MALDI MS results shows ion fragment clusters to three units with ion abundance results consistent with the presence of tin atoms within the ion fragment clusters.

Bond scission of the polymers occurs at the heteroatoms and the ethylene oxide moiety of the thiamine but the thiamine ring system remains intact. The polymers all exhibit inhibition of the cancer cell lines including two breast, two pancreatic, prostate, and colon cancer cell lines at levels lower than those for the standard cisplatin. When employing CI_{50} values as the measure, none of the tested compounds, including cisplatin, exhibits outstanding values with the CI_{50} values for the polymers more favorable than those for cisplatin.

REFERENCES

1. M. Hoch, Appl. Geochem. 16, 719 (2001)
2. C. Carraher, *Vol. 4. Group IVA Polymers* (Wiley: Hoboken, NJ, 2005)
3. A. Zhao, K. Shahi, M. Roner, G. Barot, T. Fiore, C. Pellerito, M. Scopelliti, L. Pellerito, C. Carraher, J. Polym. Mater. 25, 87 (2008)
4. C. Carraher, M. Roner, J. Organomet. Chem. 751, 67 (2009)
5. A. Nebbia, M. Dacasto, L. Ceppa, M.Gennaro Soffietti, P. Spinelli, V. Bergo, P. Di Simplicio, Vet. Res. Commun. 21, 117 (1997)
6. D. Siegmann-Louda, C. Carraher, *Vol. 3. Biomedical Applications* (Wiley, Hobokin, NJ, 2004)
7. Y. Luo, G. D. Prestwich, Curr. Cancer Drug Targets 2, 209 (2002)
8. M. Maeda, Bioconjuate Chem. 21, 797 (2010)
9. J. Fang, H. Nakamura, H. Maeda, Adv. Drug Delivery Revs 63, 136 (2011)
10. V. Torchilin, Adv. Drug Delivery Revs. 63, 131 (2011)

11. P. Goddard, I. Williamson, J. Brown, L. E. Hutchinson, J. Nicholls, K. Petrak, J. Bioactive Compatible Polymers 6, 4 (1991)
12. P. C. Phillips, Neurologic Clinics 9, 383 (1991)
13. D. Siegmann-Louda, C. Carraher, F. Pflueger, J. Coleman, S. Harless, H. Luing, Polym. Mat. Sci, Eng. 82, 83 (2000)
14. K. Abdellaoui, M. Boustta, H. Morjani, M. Manfait, M. Vert, Journal of Drug Targeting 5, 193 (1998)
15. P. A. Vasey, S. B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A. H. Thomson, L. S. Murray, T. E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, J. Cassidy, Clinical Cancer Research 5, 83 (1999)
16. M. K. Chourasia, S. K. Jain, Drug Delivery 11, 129 (2004)
17. E. Fournier, C. Passirani, N. Colin, P. Breton, S. Sagodira, J. P. Benoit, Europ. J. Pharm. Biopharmac. 57, 189 (2004)
18. K. Ulbrich, V. Subr, Adv. Drug Delivery Revs. 56, 1023 (2004)
19. *Physician's Desk Reference, 66th Edition* (Medical Economics, Montvale, NJ, 2012)
20. P. WQ. Morgan, *Condensation Polymers by Interfacial and Solution Methods* (Wiley, New York, 1965)
21. F. Millich, C. Carraher, *Interfacial Synthesis* (Dekker, New York, 1977)
22. F. Millich, C. Carraher, *Interfacial Synthesis Vol. II* (Dekker, New York, 1977)
23. C. Carraher, J. Preston, *Interfacial Synthesis* (Dekker, New York, 1977)
24. G. F. Combs, *The vitamins: Fundamental Aspects in Nutrition and Health, 3rd Ed.* (Ithaca, NY, Elsevier Academic Press, 2008)

25. M. E. Webb, A. Marquet, R. R. Mendel, F. Rebeille, A. G. Smith, Nat. Prod. Rep. 24, 988 (2007)

26. L. Bettendorff, F. Mastrogiacomo, S. J. Kish, T. Grisar, J. Neurochem. 66, 250 (2007)

27. M. Frederich, D. Delvaux, T. Gigliobianco, M. Gangolf, G. Massucchelli, B. Elias, E. De Pauw, L. Angenot, P. Wins, L. Bettendorff, FEBS Journal 276, 3256 (2009)

28. A. Schellenberger, Biochim. Biophys. Acta, 1385, 177 (1998)

29. R. H. Blake, R. B. G. O'Brien, L. P. Hager, J. Biol. Chem. 257, 9605 (1982)

30. J. Casas, E. Castellano, M. Couce, J. Ellena, A. Sanchez, J. Sanchez, J. Sordo, C. Taboada, Inorg. Chem. 43, 1957, (2004)

31. C. D. Stefano, C. Foti, A. Gianguzza, M. Martino, L. Pellerito, S. J. Sammartano, Chem. Eng. Data, **41**, 511 (1966)

32. T. Fiore, C. Pellerito, A. Fontana, F. Triolo, F. Maggio, L. Pellerito, A. Cestelli, I. Di Liegro, Appl. Organomet. Chem. **13**, 705 (1999)

33. H. J. Berg, G. Challa, U. K. Pandit, Reactive Polymers **11**, 101 (1989)

34. H. J. Berg, G. Challa, U. K. Pandit, J. Mol. Cats. **51**, 1 (1989)

35. I. Utsumi, T. Ida, K. Noda, K., *Japanese Patient 42012271* (1967)

36. I. Utsumi, T. Ida, K. Noda, K., *Japanese Patient 41014653* (1967)

37. M. Barber, R. S. Bordoli, R. D. Sedwick, A. N. Tyler, Nature **293**, 270 (1981)

38. L. K. Liu, K. L. Busch, R. G. Cooks, Analyt. Chem. **53**, 109 (1981)

39. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass. Spectrom. **2**, 151 (1988)

40. M. Karas, F. Hillenkamp, Anal. Chem. **60**, 2299 (1988)

41. C. Carraher, G. Barot, A. Battin, J. Polym. Mater. **26**, 17 (2009)

42. C. Carraher, T. Sabir, C. L. Carraher, J. Polym. Mater. **23**, 143 (2006)

43. C. Carraher, T. Sabir, C. L. Carraher, *Inorganic Organometallic Macromolecules,* (Springer, NY, 2008)

44. C. Carraher, M. R. Roner, K. Shahi, Y. Ashida, G. Barot, J. Inorg. Organomet. Polym. **18**, 180 (2008)

45. C. Carraher, M. R. Roner, K. Shahi, G. Barot, Materials **4**, 801 (2011)

46. G. Barot, M. R. Roner, Y. Naoshima, K. Nagao, K. Shahi, C. Carraher, J. Inorg. Organomet. Polym. **19**, 12 (2009)

47. C. Carraher, M. R. Roner, Materials, **2**, 1558 (2008)

48. C. Carraher, M. Ayoub, M. R. Roner, A. Moric, N. Trang, J. Chinese Adv. Mat. Society **1**, 65 (2013)

49. G. Barot, M. R. Roner, Y. Naoshima, K. Nagao, K. Shahi, C. Carraher, J. Inorg. Organomet. Polym. **19**, 12 (2009)

50. M. R. Roner, K. Shahi, G. Barot, A. Battin, C. Carraher, J. Inorg. Organomet. Polym. **19**, 410 (2009)

51. M. R. Roner, K. Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin, N. Trang, M. Alhuniti, J. Inorganic Organometallic Polymeric Materials **25**, 386 (2015)

52. B. Ekwall, V. Silano, A. Paganuzzi-Stammati, F. Zucco, *Toxicity tests with mammalian cell cultures in Short-term Toxicity Tests for Non-genotoxic Effects* (Wiley, 1990)

Figures

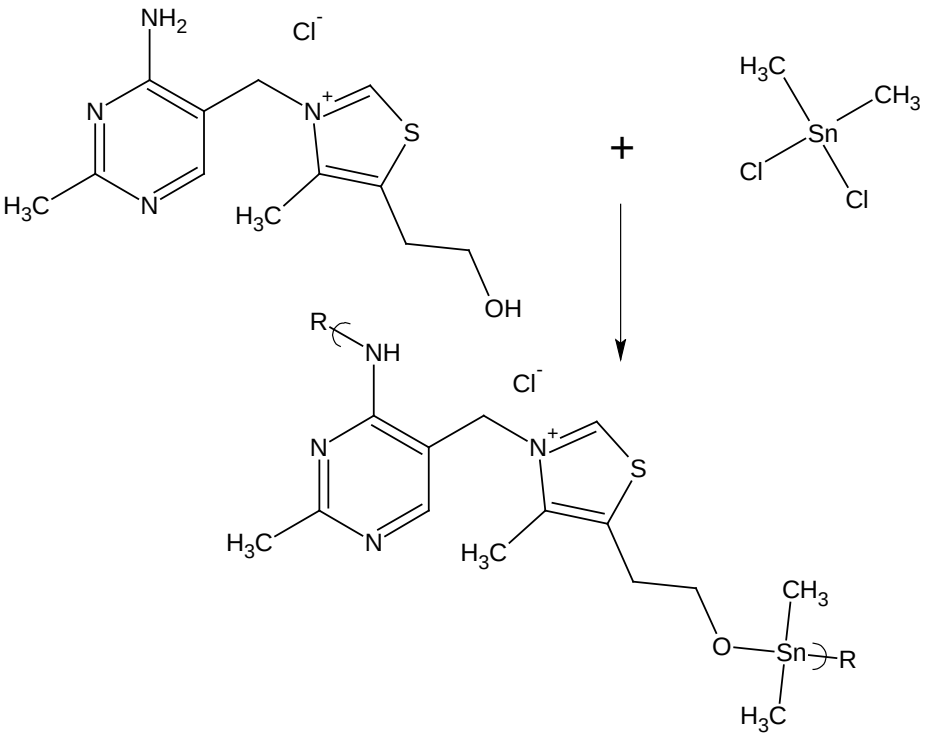


Fig. 1 Repeat unit for the product of dimethyltin dichloride and thiamine where R is simply chain extension

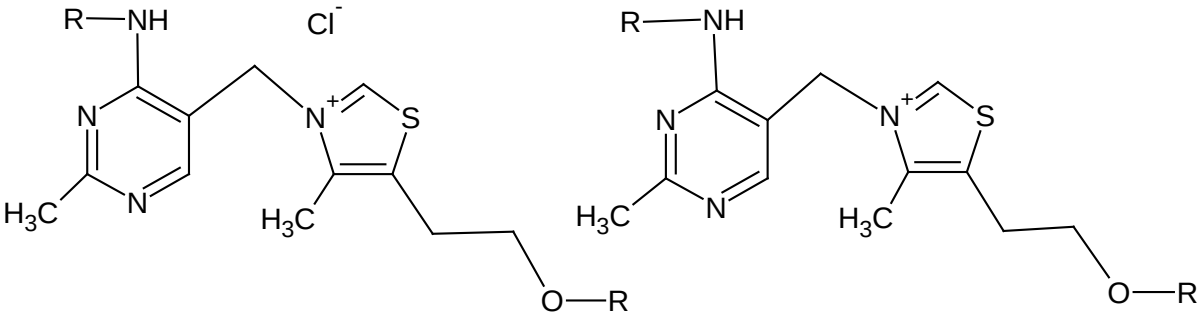


Fig 2. Structures of thiamine-containing moiety containing the chloride ion, left, and with the chloride ion absent, right.

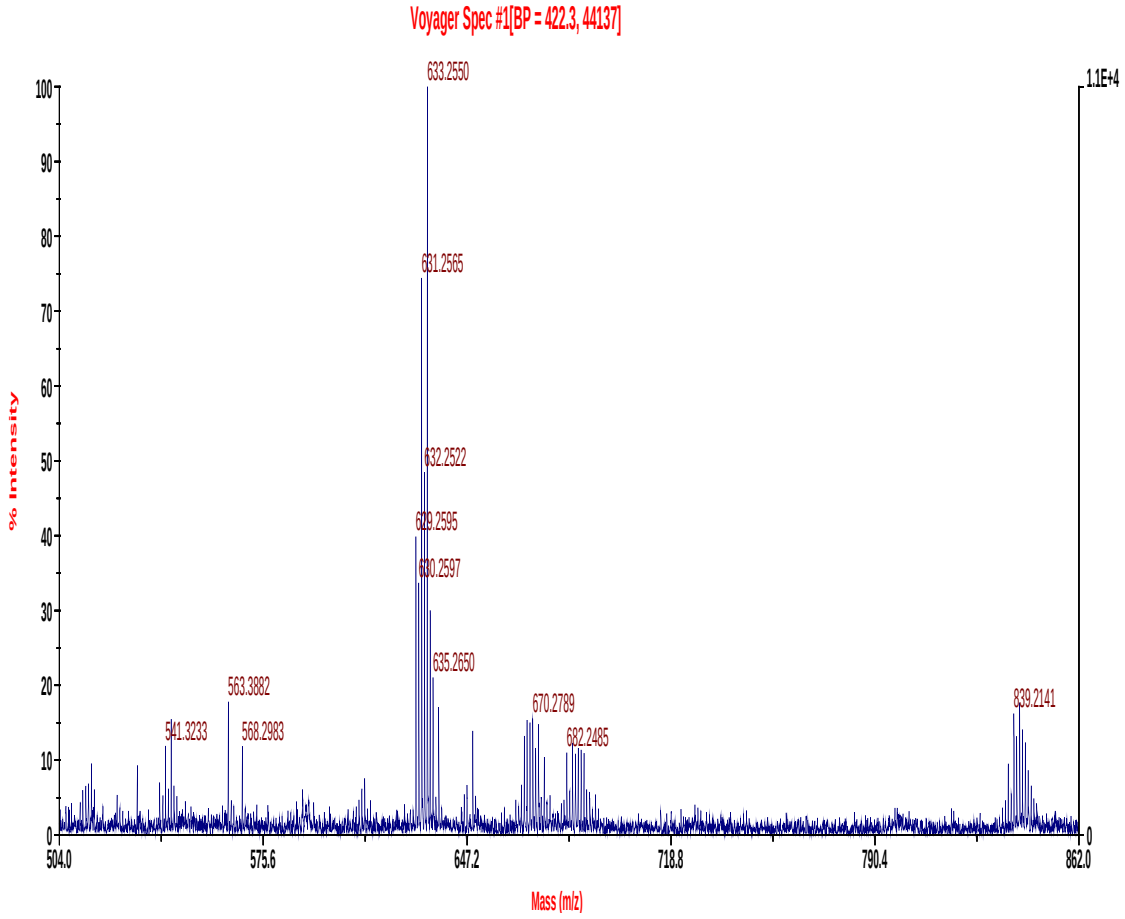


Fig 3. MALDI MS for the product of dibutyltin dichloride and thiamine obtained in the reflective mode.

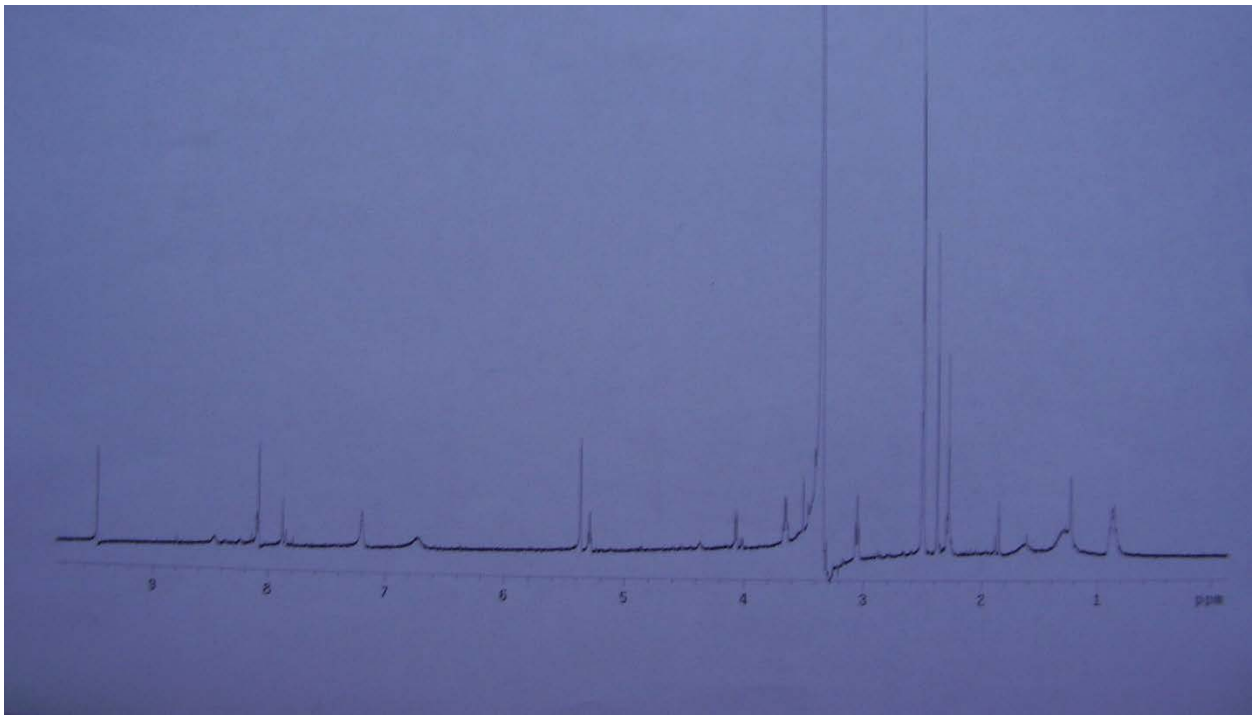


Fig. 4. Proton NMR for the product of thiamine and dibutyltin dichloride in d6-DMSO

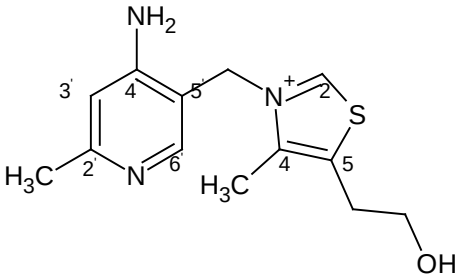


Fig 5. Thiamine structure giving the location of NMR associated protons.

Tables

Table 1 Product yield and chain length for organotin polyamine ethers from reaction of organotin dihalides with thiamine

Organotin Moiety	% Yield	Molecular Weight	Chain Length
Me_2Sn	34	1.4×10^5	280
Et_2Sn	40	4.4×10^4	86
Bu_2Sn	7	1.5×10^5	860
Ph_2Sn	26	2.2×10^5	360

Table 2 Most abundant ion fragment clusters for the product of dibutyltin dichloride and thiamine in the range of 500 to 1050 Da

Mass (Da)/Assignment		Mass (Da)/Assignment	
541	U+CH_2CH_2O	682	U+Sn,Na, CH_2CH_2O
633	U+Sn,O	841	U+Sn,2CH_2CH_2O
646	U+ Bu_2Sn,CO	1002	2U+ Bu_2Sn
670	U+Sn, CH_2CH_2O-Bu		

Table 3. Isotopic abundance matches for two tin-containing ion fragment clusters containing two tin atoms for the dibutyltin polymer. (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**U+Sn,O**	
232	12	627	10
233	13	628	11
234	43	629	42
235	35	630	35
236	94	631	93
237	51	632	51
238	100	633	100
239	35	634	35
240	81	635	70
242	32	637	32
244	22	639	22

Table 4 Most abundant ion fragment clusters for the product of diphenyltin dichloride and thiamine in the range of 500 to 2,000 Da

Ion Fragment Cluster, Da	Tentative Assignment	Ion Fragment Cluster, Da.	Tentative Assignment
588	U+ CH_2CH_2O	677	U+Sn,Na
754	U+PhSn,Na	881	U+Ph_2Sn,Na, CH_2CH_2O
924	2U-2Ph	1044	2U-NH, CH_2CH_2O
1084	2U+O	1135	2U+Na, CH_2CH_2O
1214	2U+Sn,O	1404	2U+Ph_2Sn,NH, CH_2CH_2O
1427	2U+Ph_2Sn,NH, CH_2CH_2ONa	1801	3U+Ph_2Sn

Table 5 Isotopic abundance matches for two tin-containing ion fragment clusters containing two tin atoms (Only ion fragments >5% relative abundance are reported.)

Known for Sn		U+Ph_2Sn,Na, CH_2CH_2O		2U-NH, CH_2CH_2O	
232	12	875	13	1038	13
233	13	876	15	1039	14
234	43	877	44	1040	44
235	35	878	36	1041	35
236	94	879	92	1042	94
237	51	880	53	1043	58
238	100	881	100	1044	100

239	35	882	34	1045	34
240	81	883	78	1046	81
242	32	885	32	1048	33
244	22	887	24	1050	22

Table 6 Major ion fragment clusters for the product of diethyltin dichloride and thiamine in the range of 500 to 1500 Da

Mass (Da)/Assignment		**Mass (Da)/Assignment**	
558	U+Sn	920	U+ CH_2CH_2O
577	U+Sn,O	1092	U+Th-CH_2CH_2O
682	U+Sn, CH_2CH_2O,Na	1275	3U- CH_2CH_2O
729	U+Th,Na	1493	3U+Sn

Table 7 Infrared assignments for the monomers and polymers produced from reaction of TH and dibutyltin dichloride and diphenyltin dichloride

Band Assignment	**Bu_2SnCl_2**	**Thiamine**	**Bu_2Sn Polymer**	**Ph_2SnCl_2**	**Ph_2Sn Polymer**

CH Aromatic St		3010	3010	3068,3051	3064,3046,3016
CH Aliphatic St	2960,2927	2924	2953,2930,2925		2924,2920
CH_2 Asym St	2872,2858	2862	2870,2855		2862
C=C,C=N St		1669	1651		1645
Pyrimidine Ring Breathing		1549	1601		1550
Sn-Ph St				1480	1481
CH_3Sym St	1463		1465		
C=C St				1432,1332	1428,1331
CH_3 Aym St	1380		1375		
CH_2 St	1254	1287	1286,1262		1290
Sn-N St			1152		1150
Sn-O-C St			1001		1022
Ring Breathing				996	998
CH_3 Rocking	878		882		
Sym OP Bend ring Hydrogens				729	728
Asy OP Bend Ring Hydrogens				691	691
Sn-C Asym St	592		594		
Sn-C Sym St	509		510		

Table 8 Cell line Characteristics and Identification

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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Table 9 EC_{50} values (micrograms/mL) for the tested cell lines for thiamine (TH) and the tin-containing monomers and polymers (Values given in () are the standard deviations.)

Compound	WI-38	NIH/3T3	AsPC-1	PANC-1	PC-3	*MDA	HT-29	MCF-7
Me_2SnCl_2	0.22(.1)	0.43(.1)	0.71(.1)	0.80(.1)	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)
Me_2Sn/TH	0.50(.1)	0.54(.1)	0.77(.1)	0.69(.1)	0.65(.1)	0.50(.1)	0.65(.1)	0.61(.1)
Et_2SnCl_2	0.20(.009)	0.46(.038)	0.90(.08)	0.84(.1)	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)
Et_2Sn/TH	0.55(.1)	0.33(.1)	0.44(.1)	0.38(.1)	0.43(.1)	0.62(.1)	0.78(.1)	0.90(.1)
Bu_2SnCl_2	0.20(.05)	0.0035(.005)	>0.15	>0.15	1.4(.11)	1.4(.12)	1.2(.11)	0.70(.06)
Bu_2Sn/TH	0.45(.1)	0.60(.1)	0.80(.1)	0.80(.1)	0.55(.1)	0.70(.1)	0.70(.1)	0.75(.1)
Ph_2SnCl_2	0.25(.1)	0.66(.16)	0.83(.13)	0.71(.1)	0.82(.1)	0.76(.1)	0.56(.1)	0.59(.1)
Ph_2Sn/TH	0.45(.1)	0.35(.1)	0.67(.1)	0.74(.1)	0.33(.1)	0.40(.1)	0.51(.1)	0.43(.1)
TH	>60	>60	>60	>60	>60	>60	>60	>60
Cisplatin	0.05(.04)	1.2(.019)	1.4(.15)	0.34(.012)	1.0(.1)	1.0(.1)	2.0(.21)	3.0(.28)

*MDA = MDA MB-231

Table 10 CI_{50} values for the tested cell lines for thiamine, TH, and the tin-containing monomers and polymers using WI-38 (top) and NIH 3T3 (bottom) as standards

Compound	WI-38/ WI-38	WI-38/ NIH/3T3	WI-38/ AsPC-1	WI-38/ PANC-1	WI-38/ PC-3	WI-38/ *MDA	WI-38/ HT-29	WI-38/ MCF-7
Me_2SnCl_2	1	0.51	0.31	0.28	0.43	0.50	0.39	0.33
Me_2Sn/TH	1	0.93	0.65	0.56	0.77	1.0	0.77	0.82
Et_2SnCl_2	1	0.43	0.22	0.24	0.33	0.31	0.28	0.26

Et_2Sn/TH	1	1.7	1.3	1.5	1.3	0.89	0.71	0.61
Bu_2SnCl_2	1	1.0	--	--	0.14	0.14	0.17	0.29
Bu_2Sn/TH	1	0.75	0.56	0.56	0.82	0.64	0.64	0.60
Ph_2SnCl_2	1	0.37	0.30	0.35	0.30	0.32	0.45	0.42
Ph_2Sn/TH	1	1.3	0.67	0.61	1.4	1.1	0.88	1.1
Cisplatin	1	0.42	0.36	0.15	0.05	0.05	0.03	0.02

*MDA = MDA MB-231

Compound	NIH/3T3/ WI-38	NIH/3T3/ NIH/3T3	NIH/3T3/ AsPC-1	NIH/3T3/ PANC-1	NIH/3T3/ PC-3	NIH/3T3/ *MDA	NIH/3T3/ HT-29	NIH/3T3/ MCF-7
Me_2SnCl_2	2.0	1	0.61	0.54	0.84	0.98	0.77	0.65
Me_2Sn/TH	1.1	1	0.70	0.78	0.83	1.1	0.83	0.89
Et_2SnCl_2	2.3	1	0.51	0.55	0.75	0.72	0.65	0.60
Et_2Sn/TH	0.60	1	0.75	0.87	0.76	0.53	0.42	0.37
Bu_2SnCl_2	1.0	1	--	--	0.14	0.14	0.17	0.29
Bu_2Sn/TH	1.3	1	0.75	0.75	1.1	0.86	0.86	0.80
Ph_2SnCl_2	2.6	1	0.80	0.93	0.80	0.87	1.2	1.1
Ph_2Sn/TH	0.78	1	0.52	0.47	1.0	0.88	0.68	0.81
Cisplatin	24.	1	0.86	3.5	1.2	1.2	0.60	0.40

*MDA = MDA MB-231

CHAPTER 7

SYNTHESIS AND PRELIMINARY ANTICANCER RESULTS FOR POLYMERS FROM THE REACTION OF ORGANOTIN DIHALIDES AND THIODIGLYCOLIC ACID[5]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 12 and 13) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Pham, N.; Roner, M.R.; **Miller, L. C.;** Black, K.; and Crichton, R**.**

Carraher E. Charles Jr., Michael R. Roner, Ngoc Pham, Lindsey C. Miller, Kendra Black & Ryan Crichton (**2015**) Synthesis and Preliminary Anticancer Results for Polymers From the Reaction of Organotin Dihalides and Thiodiglycolic Acid, Journal of Macromolecular Science, Part A: Pure and Applied Chemistry, 52:10, 786-797, DOI: 10.1080/10601325.2015.1067021.

[5] Used with permission of Taylor & Francis, 2017

Synthesis and Preliminary Anticancer Results for Polymers from the Reaction of Organotin Dihalides and Thiodiglycolic Acid

Running Title: Organotin Polyesters Thiodiglycolic Acid

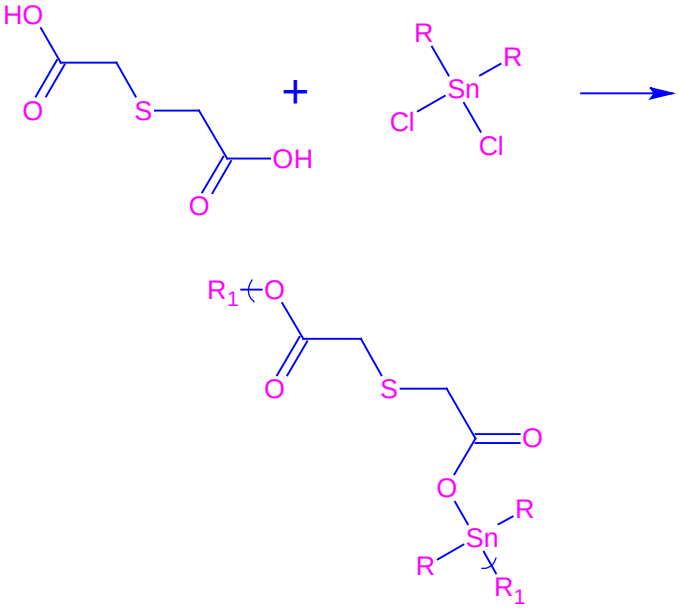
Charles E. Carraher, Jr.,[a] Ngoc Pham, [a] Michael R. Roner,[b] Lindsey C. Miller,[b] and Kendra Black, [a] Ryan Crichton[a]

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 and Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410; carraher@fau.edu

b. University of Texas at Arlington, Department of Biology, Arlington, TX 76010; roner@uta.edu

*Corresponding author: Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; fax 561-297-2759; words approximately 7,000; 24 pages non-formatted; 45 pages formatted.

Special Art if Required



Abstract: Organotin polyesters were synthesized in good to poor yield with yield and chain length generally decreasing as the alkyl chain on the organotin increases. MALDI MS, NMR, and IR data is consistent with the proposed repeat unit. MALDI MS ion fragment clusters to four repeat units are found. The IR shows the presence of a new band assigned to the formation of the Sn-O. With the exception of the dimethyltin polymer, all of the polymers exhibit good inhibition of a variety of cancer cell lines including two breast cancer, prostate, two pancreatic cancer and colon cancer cell lines.

Key Words: Organotin polymers, organotin polyesters, pancreatic cancer, MALDI MS, interfacial polycondensation, prostate cancer, breast cancer

1. Introduction

The biological activity of organotin compounds has been known for well over 100 years. For over 80 years organotin compounds have been known to inhibit cancer cell growth (1-3). More organotin compounds are available commercially than any other metal-containing organometallic (1-3). Further, more organotin compounds have undergone testing as potential anticancer agents than any other single group of compounds (1-4).

Much of our recent effort focused on the synthesis and biological characterization of organotin polymers. Many of them have arrested the growth of a number of cancer cell lines including those associated with lung, bone, colon, breast, ovarian, and prostate cancer and most recently, pancreatic cancer cell lines. These products generally exhibit good inhibition of cell growth equal to and greater than cisplatin, one of the most widely employed chemotherapeutic drugs. This has been recently reviewed (4).

The mechanism(s) of action of tin-containing compounds is not well-understood and has been recently summarized as has the advantages of employing polymeric drugs (2-18). The locations of activity are numerous and these multiple sites may be advantageous in fighting cancer since they may allow curtailment of cancer growth through several mechanisms (4).

Much of our present syntheses have been driven by the idea that coupling the biological activity of the organotin moiety with that of Lewis bases that also exhibit biological activity results in compounds that are more likely to give the desired anticancer and antiviral activities (4).

For the present study, the biologically active Lewis base is thiodiglycolic acid, TA (Fig. 1.), also known as thiodiacetic acid mercaptodiacetic acid and dicarboxydimethyl sulfide (19). It is a diacid belonging to a large range of physiological products of human metabolism. It is found at concentrations less than 20 mg/L in the urine of healthy individuals (19). Thiodiglycoid acid, TA, concentration increases when humans are exposed to changes affecting our redox equilibria. TA is a metabolite of the warfare chemical mustard gas. TA is a major metabolite of the anticancer drug ifosfamide. It is a very potent drug and shuts down the mitochondria and sequesters or inhibits carnitine (19).

There are no reports of the direct incorporation of thiodiglycolic acid forming traditional condensation polymers. Mathias and coworkers reported the use of thio-containing diols from thiodiglycolic acid with subsequent incorporation of these diols into various polymers as part of an effort to incorporate sequential thioethylene units into copolymers (20,21).

The thiodiglycolic acid, TA, moiety has been incorporated into a variety of polymers for different reasons. Li and coworkers created a class of high intensity focused ultrasound and redox dual response polymer micelles using a weak disulfide bond linkage employing TA (22). Prussian blue nanoparticles were stabilized using a pi-congated polymer again using TA (23). Donor-type acceptor conjugated polymers were formed using TDGA as a starting material (24). Henckens and

coworkers synthesized 3,4-diphenyl-substituted poly(thienylene vinylene) low-band-gap polymers through the dithiocarbamate route employing TDGA as one of the reactants (25).

Further, a number of coordination polymers have been formed including lanthanide and terbium coordination polymers (26,27).

We recently described the synthesis of Group VA polyesters from the reaction of triphenylmetal dihalides with the salt of TA (Fig 2.) (28).

Here we report the initial synthesis of organotin polyesters derived (Fig. 3) from the reaction of the organotin dihalide with the salt of thiodiglycoid acid and the ability of the products to inhibit the growth of a variety of cancer cells.

The interfacial condensation process was popularized by Paul Morgan and co-workers at DuPont (29) and by Carraher and coworkers (30-32). For the synthesis of our polymers we employ the rapid (generally within 15 seconds) interfacial condensation of diorganotin dihalides with TA. Since the reactants are commercially available and the interfacial condensation system is employed industrially to synthesize aramids and polycarbonates the current polymers can be readily synthesized on a small to large scale.

2. Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the Lewis base, here thiodiglycolic acid, (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 ^{o}C). Stirring was begun and a heptane solution (30 ml) containing the organotin dihalide (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 to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

The reactants (diphenyltin dichloride (1135-99-5), dimethyltin dichloride (753-73-1), 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), and dicyclohexanetin dibromide (2954-94-1), dibenzyltin dichloride were obtained from Ventron Alfa Inorganics, Beverly, Mass; and thiodiglycolic acid (123-93-3) from Acros Organics) were used as received.

2.2 Physical Characterization

Light scattering photometry was in carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 and DMSO polymer solutions. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet

iS5 FTIR fitted with an ATR id5. ^{1}H NMR spectra were obtained in d6-DMSO 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 2000 to 100,000. Fifty to two hundred shots were typically taken for each spectrum. Results employing alpha-cyano-4-hydroxycinnamic acid are included in the present paper. The solid product, along with solid matrix, were mixed together employing copper spheres giving a fine powder that was employed to obtain the spectra.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human pancreas adenocarcinoma cell line (AsPC-1), human pancreas epithelioid duct carcinoma cell line (PANC-1) or mouse embryo-fibroblast (NIH/3T3) cell line. Following a 24 h incubation period, the text compounds were added 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. 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 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.

3. Results and Discussion

3.1 Yield and Chain Length

The reaction between thiodiglycolic acid and organotin dihalides is general occurring for all organotin reactants attempted. Results are given in Table 1.

Yield ranges from poor to good (2 to 91%). For the linear alkyl organotins, yield generally decreases as the length of the alkyl group increases consistent with bulkiness being important in creating product. The products are white as expected. They vary in tackiness with tackiness increasing as the length of the dialkyl chain increases. Thus, the product from dimethyltin dichloride is not tacky while the product from the dibutyltin dichloride is quite tacky.

Table 1 also contains the molecular weights and chain lengths for the products. The products range from being high polymers (diphenyltin) to short polymer chains (dicyclohexyltin and dioctyltin). The longest alkyl chains have the lowest chain length consistent with steric hindrance

of the alkyl grouping playing a role in the polymerization process. This is similar to that found for product yield.

3.2 Infrared Spectral Results

Infrared spectral analysis was carried out for all of the samples over the range of 4000-650 cm^{-1}. All band locations are given cm^{-1}. Tables 2 and 3 contain results for the dibutyltin and diphenyltin samples.

Infrared spectral analysis is consistent with the proposed structure and with other reported analyses (4, 33-35). All spectra show bands characteristic of both reactants. For instance for C-H stretching, the dialkyltin polymers have both bands characteristic of the dialkytin moiety just below 3000 derived from both the alkyltin moiety and from the TA moiety. For the diphenyltin-derived polymer, bands are present above 3000 characteristic of the diphenyltin moiety and below 3000 characteristic of aliphatic C-H st derived from the TA moiety. A new band for the product is found and assigned to the Sn-O linkage at about 1020.

The polyesters can exist as bridging or distorted octahedral and non-bridging or distorted tetrahedral about the tin (4, 33-35) (Fig. 4). Infrared spectroscopy is the easiest way to determine the structure about the tin. Bridging asymmetric carbonyl absorptions are found around 1570 (all infrared bands are given in cm^{-1}). The bridging symmetric carbonyl band is found around 1410-1430. Non-bridging asymmetric carbonyl bands are found about 1600-1650; and the corresponding symmetric carbonyl band found about 1350-1390.

Table 4 contains results for all of the products with respect to bands associated to bridging/non-bridging. All but one of the products show a mix of bands consistent with the structures consisting of both bridged and non-bridges geometries about the tin atom. It is of

interest that the only polymer not to be a mixture of bridging and non-bridging is the most steric hindered, the dioctyltin product. It is reasonable that the non-bridged structure, which is what is indicated for the dioctyltin product, is less steric demanding in comparison to the octahedral bridging structure.

3.3 Proton NMR

NMR spectra were taken of the materials. Thiodiglycolic acid shows two singlets at 3.37 and 3.68 (all bands given in ppm) derived from the methylene protons. The polymer derived from dibutyltin dichloride has bands at 3.37 and 3.69 from the thiodiglycolic acid derived moiety and a doublet at 1.57 and 1.55; singlet at 1.44, a quartet at 1.29, 1.27, 1.25 and 1.23 and a triplet at 0.85, 0.83 and 0.81 consistent with the presence of the dibutyltin moiety. The product from diphenyltin dichloride has bands at about 3.40 and 3.69 from the thiodiglycolic acid moiety and bands at 7.85 and 7.40 derived from the diphenyltin moiety. Thus, proton NMR spectroscopy is consistent with the presence of units derived from both reactants. Because of the poor solubility of the polymers, additional implications from the data are not confidently derived.

3.4 MALDI MS

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. Matrix-assisted desorption/ionization was independently introduced in 1981 by Barber and Liu and coworkers (36,37). The addition of the laser as the energy source was introduced by Tanaka, Hillenkamp and coworkers in 1988 (38,39). The combinations of these concepts allowed

the creation of matrix-assisted laser/desorption mass spectroscopy, MALDI MS. We have been using a modification of this technique that allows MALDI MS to be obtained on non-volatile and insoluble products. This approach has been recently reviewed (40-42).

MALDI MS was carried out on the products over the general range of 500 to 1100 Da. All mass values are given in Da.

A portion of the MALDI MS for the dibutyltin polymer is given in Figure 6. Each of the ion fragment clusters above 500 (all ions are given in daltons, Da, or m/e =1) are actually clusters of ions that are produced because of the presence of tin atom(s) within each cluster. Because tin has isotopes, different ion fragments are created that have the same structural formula but vary by the particular tin isotope present. This creates what is often referred to as spectral "fingerprints" characteristic of the natural abundance of these isotopes. Most of the fragments given in the following tables are actually clusters of such ion fragments. Along with the pictorial representations as given in Figure 5, such distributions can also be represented in table form and compared with known values. Table 5 contains the most abundant ion fragment clusters given in Figure 5. The following abbreviations are utilized to describe the tentative ion fragment cluster assignments as follows: U = one unit, 2U = two units; T = thiodiglycolic acid minus two hydrogens; Bu = butyl moiety, O = oxygen. Additional less abundant ion fragment clusters are also present. Sodium is a common contaminant.

Ion fragment clusters to two and a half are found.

There is loss of butyl groups from the organotin moiety. This is common for organotin compounds since they are not stable to the radiation of the laser employed in the MALDI MS (40-42).

As noted before, isotopic abundance matches are employed to support the presence of tin atoms in the isotopic fragment clusters. Tin contains ten isotopes of which seven are considered significant. At higher masses, isotope matches are difficult because of the low intensities of generated ion fragments. Even so, at lower masses such isotope matches are possible. Table 6 contains two isotopic matches for ion fragment clusters containing a single tin atom and Table 7 contains two matches for ion fragment clusters containing two tin atoms. The abundance matches are consistent with the presence of one and two tin atoms within the particular ion fragment clusters.

The isotopic matches are in reasonable agreement with the standard and consistent with the presence of one and two tin atoms in the fragment clusters. The “mildness” of MALDI MS is shown by the lack of fragmentation of the TA moiety.

Major ion fragment clusters for the product of dimethyltin dichloride and TA are given in Figure 6 and Table 8.

Ion fragment clusters containing four and a half repeat units are found.

Ion fragment isotopic abundance values are given for two ion fragment clusters containing two tin atoms and one cluster containing three tin atoms (Table 9 and 10). Again, the matches are reasonable consistent with the ion fragment clusters containing tin atoms.

In each case, ion fragment clusters are consistent with the presence of tin and that tin and the oxygen-associated sites are the sites for bond scission. Presumably, the ion fragment clusters are produced through the bond scission of the larger polymer chain at these sites resulting in the formation of these lower mass ion fragment clusters. Further, loss of alkyl and aryl moieties on the tin is also the result of this preferential instability of the organotin moiety at sites of bond scission. These preferential sites of bond breakage are shown in Figure 7

3.5 Cancer Cell Lines

Much of our recent activity has focused on the synthesis and preliminary cancer cell line testing of various organotin-containing polymers. In general, as the organotin dihalide is varied for a particular Lewis base, the polymer containing the dibutyltin moiety shows the greatest ability to curtail cancer cell line growth followed by those containing the diphenyltin moiety (4). The next greatest activities were by dipropyltin, diethyltin, and dimethyltin-containing polymers. The lowest activity was generally found for long chain alkyl groups such as dioctyltin. This activity was found for a variety of cancers (4).

Table 11 contains the cell lines employed in the current study.

While different measures have been employed in the evaluation of cell line results the most widely employed involves the concentration, dose, needed to reduce the growth of the particular cell line. Here we will employ the term effective concentration, EC. 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 here is given the symbol EC_{50}. Table 12 contains the EC_{50} values found for the monomers and polymers and include values for cisplatin as a standard.

There are a number of observations from the data presented in Table 12. First, the polymers have a much greater toxicity towards the cancer cell lines compared with TA, which can be considered nontoxic over the studied range. The polymers generally have a greater toxicity than cisplatin towards the tested cell lines showing EC_{50} values less than cisplatin. Some of the EC_{50} values for the polymers are in the nanogram/mL range. Second, the polymers and monomer are both toxic towards all of the tested cell lines (with the exception of the dimethyltin polymer). In general, the dimethyltin polymer is the least toxic towards the cancer cell lines while the other polymers exhibit decent ability to inhibit cancer cell growth.

Several observations with respect to individual cell lines results are appropriate. 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 polymers we found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure (4,43,44). In these studies polymers containing a Lewis base that possesses the O-Phenylene moiety, such as hydroquinone and hydroquinone derivatives (45) and diethylstilbestrol (44), exhibits a relatively 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 TA in the present study, that do not contain this structural moiety showed little difference between the ability to inhibit the two cell lines (4). Here, there is little difference between the ability to inhibit the two breast cancer cell lines with the various organotin polymers.

One of our recent focuses regards pancreatic cancer. Pancreatic cancer afflicts close to 32,000 individuals each year in the United States and 168,000 worldwide, and nearly all patients die from the ravages of their disease within 3 to 6 months after detection. 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). Treatment of pancreatic cancer is rarely successful as this disease typically metastasizes prior to detection. There is no chemotherapy for metastasized pancreatic cancer. We recently described the ability of a number of organotin polymers to inhibit pancreatic cancer (4,46-49). Because pancreatic cancer does not have a generally accepted "cure" one of our current focuses is on the synthesis of materials that might be successful in combating pancreatic cancer. 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. Both are human cell lines and are widely employed in testing for inhibition of pancreatic cancer. For the current study, the dibutyltin, dicyclohexane and diphenyltin polymers show good inhibition of both cell lines with the inhibition sufficient to indicate these three polymers should considered for future studies related to inhibition of pancreatic cancer.

The PC-3 results are of interest because this particular prostate cell line is viewed as the most resistant of the prostate cancer cell lines (50). The dibutyltin, dicyclohexyltin and diphenyltin

polymers all exhibit good inhibition of the PC-3 cells equal to or better than that for the control cisplatin. Towards the PC-3 the EC_{50} value for dibutyltin dichloride is 1.4, for cisplatin it is 1.0 and for the corresponding polymer the EC_{50} is 0.0014.

Third, the overall general trend with respect to the polymers is Bu>Cy>Ph>Et>Me which is approximately what is generally found for a variety of series of organotin polymers with respect to Bu and Ph being among the most toxic towards the cancer cell lines. It is fortunate that it is the dibutyltin moiety that generally gives the best anticancer activity for several reasons (1,4). First, it is the least expensive of the organotin halides and available in the ton and greater quantities. Second, it is the least toxic to humans of the lower alkyltins. Third, commercially it is the most widely employed organotin moiety (1).

For this set of polymers it appears that activity increases as the size of the alkyl group increases maximizing for the butyltin product. It is not know if this trend is due to the ability of this product to penetrate the cancer cell walls or for some other reason. It is known that the polymers themselves inhibit cancer growth and not some break-down product. Further, it is known that the organotin polymers are cytotoxic and cell death is by necrosis.

Another measure of the potential use of compounds 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, we will simply employ the term chemotherapeutic index, CI, so that the CI_{50} is then the ratio of the EC_{50} for the NIH/3T3 or WI-38 cells divided by the EC_{50} for the particular test cell. These results are shown

employing WI-38 and NIH/3T3 cells as the standards in Table 13. 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. In the present case, none of the polymers, monomers or cisplatin (except for several cell lines employing NIH/3T3 cells as standards) shows values greater than one.

There is not agreement as to which measure, EC_{50} or CI_{50} values, is the best measure of the ability of tested materials to inhibit cancer growth so results employing both measures are presented.

A second study is superimposed onto the current study. This second study involves comparing results from the two most frequently used cell lines as standards involved in the evaluation of the effectiveness of compounds through calculating CI values. These two cell lines are the NIH/3T3 and WI-38 cell lines.

NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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. While NIH/3T3 cells are often favored because of ease of handling aided by an infinite life span, results from WI-38 cells are given greater importance when there is a difference (51).

To see if the more easily handled NIH/3T3 cells can be used in place of the WI-38 cell one can simply observe the ratio of EC_{50} for the WI-38 values divided by the EC_{50} for the NIH/3T3. If there is no difference the ratio should be about 1. In the present case, the values range from 0.08 to 6 so there is a significant difference. Next, it should be determined if any of the differences would eliminate compounds from further study. In the present study this is not the case. The only major difference is with the dibutyltin products where the WI-38 results give values generally significantly less than one, while the NIH/3T3 values are greater than two for the PC-3, breast cancer cell lines, and the HT-29 cell line. In cases where there is a difference, greater confidence is given to the WI-38 results. Thus, based on CI values alone giving greater significance to the WI-38 values, no products would be eliminated that should not be eliminated.

In other studies we have found mixed results where the used of 3T3 cells as the standard included samples that might otherwise be eliminated (4). Since greater confidence is given to results obtained when WI-38 cells are used, when possible they are preferred over the use of NIH/3T3 cells (51).

As noted before, we recently synthesized Group VA polyesters similar to those reported here for the organotin polyesters (28). The arsenic polymer exhibits outstanding inhibition of the AsPC-1 adenocarcinoma cell line with a low EC_{50} and the largest CI_{50} thus far found by us for the AsPC-1 pancreatic cancer cells. Both the arsenic and antimony polymers show good inhibition of the PC-3 prostate cancer cell line. The antimony polymer showed good inhibition of both the MDA-MB-231 breast cancer cells that are estrogen-independent and estrogen receptor negative and the MCF-7 breast cancer cells that are an estrogen receptor (ER) positive cell line. Thus, all of the polymers exhibit decent inhibition of at least some of the cancer cell lines.

By comparison, the organotin polymers from dibutyltin, dicyclohexyltin and diphenyltin exhibited lower EC_{50} levels than the Group VA polyesters. For instance, the EC_{50} for the AsPC-1 for the arsenic polymer was 0.055; for the bismuth TA polymer it was 20; but the EC_{50} values for the dibutyltin AT polymer was 0.054, diphenytin 0.14 and for the dicyclohexyltin it as 0.0015. Thus, the dibutyltin, dicyclohexyltin and diphenyltin TA polymers generally showed lower EC_{50} values compared with the Group VA TA polymers. By comparison, the Group IVA TA polymers often showed much larger CI_{50} values. Considering only CI_{50} values based on WI-38 cells as the standard, the organotin polymers did not show any values greater than one, but the Group IVA polymers often exhibited large values. For instance, for the MDA MB-231 breast cancer cell line, the CI_{50} value for As was >73, for Sb it was >42000 and for Bi it was 1.5. The reason for this difference is that the Group VA polymers were much less toxic to the WI-38 standard compared with the organotin TA polymers.

As noted before, there is not agreement as to whether the EC_{50} or CI_{50} results are the most important in indicating which compounds are the best for further testing. Here, the CI_{50} values for the Group VA polymers are superior to the CI_{50} values for the organotin polymers, but the EC_{50} values for the organotin polymers are generally lower compared with the Group VA polymers. Thus, both groups have members that merit further testing, but based on different measuring criteria.

4. Conclusions

Organotin polyesters were synthesized from the interfacial polycondensation of organotin dihalides and thiodiglycolic acid. The products are formed rapidly, less than 15 seconds, in poor to good yield.

The products range from being low to high polymers. Yield and chain length generally decreases as the steric nature of the alkyl substituent on the tin increases. Infrared spectral analysis is consistent with the proposed structure. Infrared spectral analysis shows the presence of the Sn-O linkage and is consistent with the geometry about the tin being generally a mixture of tetrahedral or non-bridging and octahedral or bridging. MALDI MS shows the formation of ion fragment clusters to four units. Isotopic abundance results are consistent with the ion fragment clusters containing tin. Cell line results based on EC_{50} values the polymers containing the dibutyltin, dicyclohexane, and diphenyltin moieties show good inhibition of the various cancer cell lines with results consistent with further testing being merited. In fact, these are in the nanogram/mL range and constitute some of the lowest thus far found (4). When employing CI_{50} values as the measure, none of the tested compounds, including cisplatin, exhibits outstanding values.

References

1. Hoch, M. (2001) Appl. Geochem., 16, 719-743.
2. Carraher, C., Vol. 4. Group IVA Polymers, Wiley: Hoboken, NJ, 2005.
3. Zhao, A., Shahi, K., Roner, M., Barot, G., Fiore, T., Pellerito, C., Scopelliti, M., Pellerito, L., Carraher, C. (2008) J. Polym. Mater., 25, 87-109.
4. Carraher, C., Roner, M., J. (2014) Organometallic Chem, 751, 67-82.
5. Nebbia, C., Dacasto, M., Ceppa, L., Gennaro Soffietti, M., Spinelli, P., Bergo, V., Di Simplicio, P. (1997) Vet. Res. Commun., 21, 117-125.
6. Siegmann-Louda, D., Carraher, C. Vol. 3. Biomedical Applications, Wiley, Hobokin, NJ, 2004, 119-192.
7. Luo, Y., Prestwich, G. D. (2002) Curr. Cancer Drug Targets, 2, 209-226.
8. Maeda, M. (2010) Bioconjuate Chem., 21, 797-802.

9. Fang, J., Nakamura, H., Maeda, H. (2011) Adv. Drug Delivery Revs, 63, 136-151.

10. Torchilin, V. (2011) Adv. Drug Delivery Revs., 63, 131-135.

11. Goddard, P., Williamson, I., Brown, J., Hutchinson, L. E., Nicholls, J., Petrak, K. (1991) J. Bioactive Compatible Polymers, 6, 4-24.

12. Phillips, P. C. (1991) Neurologic Clinics, 9, 383-404.

13. Siegmann-Louda, D., Carraher, C., Pflueger, F., Coleman, J., Harless, S., Luing, H. (2000) Polym. Mat. Sci, Eng., 82, 83-84.

14. Abdellaoui, K., Boustta, M., Morjani, H., Manfait, M., Vert, M. (1998) Journal of Drug Targeting, 5, 193-206.

15. Vasey, P. A., Kaye, S. B., Morrison, R., Twelves, C., Wilson, P., Duncan, R., Thomson, A. H., Murray, L. S., Hilditch, T. E., Murray, T., Burtles, S., Fraier, D., Frigerio, E., Cassidy, J. (1999) Clinical Cancer Research, 5, 83-94.

16. Chourasia, M. K., Jain, S. K. (2004) Drug Delivery, 11, 129-148.

17. Fournier, E., Passirani, C., Colin, N., Breton, P., Sagodira, S., Benoit, J. P. (2004) Europ. J. Pharm. Biopharmac., 57, 189-197.

18. Ulbrich, K., Subr, V. (2004) Adv. Drug Delivery Revs., 56, 1023-1050.

19. Physician's Desk Reference, 66th Edition, Medical Economics, Montvale, NJ, 2012.

20. Muthiah, J., Johnson, C. G., Thompson, R., Mathias, L. (1995**)** Macromolecules, 28, 7796-7805.

21. Mathias, L., Canterberry, J. B. (1980) Macromolecules, 13, 1723-1724.

22. Li, Y., Tong, R., Xia, H., Zhang, H., Xuan, J. (2010) Chemical Comm., 46, 7739-7741.

23. Yamada, M., Ohnishi, N., Watanabe, M., Hino, Y. (2009) Chemical Comm., 46, 7203-7205.

24. Manjunatha, M., Adhikari, A., Hegde, P., Suchand, S., Philip, R., (2009) J. Mat. Sci., 44, 6069-6077.

25. Henckens, A., Colladet, K., Fourier, S., Cleij, T., Lutsen, L., Gelan, J., Vanderzande, D. (2005) Macromolecules, 38, 19-26.

26. Wang, X., Hou, X., Fu, F., Wang, J., Li, H., Ren, Y., Zhang, M., (2011) Wuji, Huaxue Xuebao, 27, 174-178.

27. Hou, X., Wang, X., Li, D., Fu, F., Zhao, X., Wang, Y, (2008**)** Wuji, Huaxue, Xuebao, 24, 913-918.

28. Carraher, C., Roner, M. R., Pham, N., Moric, A. (2014) J. Macromol. Sci, Part A, 51, 547-556.

29. Morgan, P. W. Condensation Polymers by Interfacial and Solution Methods, Wiley, New York, 1965.

30. Millich, F., Carraher, C. Interfacial Synthesis, Dekker, New York, 1977.

31. Millich, F., Carraher, C. Interfacial Synthesis. Vol. II., Dekker, New York, 1977.

32. Carraher, C., Preston, J. Interfacial Synthesis, Dekker, New York, 1977.

33. Carraher, C., Suresh, V., Roner, M. (2015) JCAMS, 3, 32-44.

34. Carraher, C., Truong, N. T. C., Roner, M. R., Moric-Johnson, A., Sookdeo, N., Trang, N. T. (2014) Inorganica Chimica Acta, 423 (B), 83-92.

35. Carraher, C., Morrison, A., Roner, M. R., Moric, A., Trang, N. T. (2014) J. Inorg. Organomet. Polym., 24, 182-189.

36. Barber, M., Bordoli, R. S., Sedwick, R. D., Tyler, A. N. (1981) Nature, 293, 270-275.

37. Liu, L. K., Busch, K. L., Cooks, R. G. (1981) Analyt. Chem., 53, 109-113.

38. Tanaka, K., Waki, H., Ido, Y., Akita, S., Yoshida, Y., Yoshida, T. (1988) Rapid Commun. Mass. Spectrom., 2, 151-153.

39. Karas, M., Hillenkamp, F. (1988) Anal. Chem., 60, 2299-2301.

40. Carraher C., Barot G., Battin A. (2009) J Polym Mater., 26, 17-31.

41. Carraher C., Sabir T., Carraher C. L. (2006) J Polym Mater., 23, 143-151.

42. Carraher, C., Sabir, T., Carraher, C. L., Inorganic Organometallic Macromolecules, Springer, NY, 2008, pp. 329-350

43. Carraher, C., Roner, M. R., Shahi, K., Ashida, Y., Barot, G. (2008) J. Inorg. Organomet. Polym., 18, 180-188.

44. Carraher, C., Roner, M. R., Shahi, K., Barot, G. (2011) Materials, 4, 801-815.

45. Barot, G., Roner, M., Naoshima, Y., Nagao, K., Shahi, K., Carraher, C. (2009) J. Inorg. Organomet. Polym., 19, 12-27.

46. Carraher, C., Roner M. R. (2009) Materials, 2, 1558-1566.

47. Carraher, C., Ayoub, M., Roner, M. R., Moric, A., Trang, N. (2013) JCAMS, 1, 65-73.

48. Barot, G., Roner, M. R., Naoshima, Y., Nagao, K., Shahi, K., Carraher, C. (2009) J. Inorg. Organomet. Polym., 19, 12-27.

49. Roner, M. R., Shahi, K. R., Barot, G., Battin, A., Carraher, C. (2009) J. Inorg. Organomet. Polym., 19, 410-414.

50. Roner, M. R., Shahi, K., Moric-Johnson, A., Miller, L., Barot, G., Battin, A., Trang, N., Alhuniti, M. (2015) J. Inorganic Organometallic Polymeric Materials, 25, 386-399.

51. Ekwall, B., Silano, V., Paganuzzi-Stammati, A., Zucco, F. (1990) Toxicity tests with mammalian cell cultures in Short-term Toxicity Tests for Non-genotoxic Effects, Wiley, 1990.

Figures

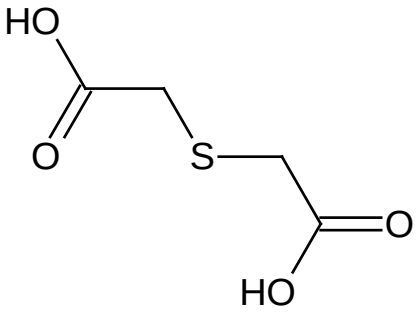


Fig. 1. Structure of thiodiglycolic acid.

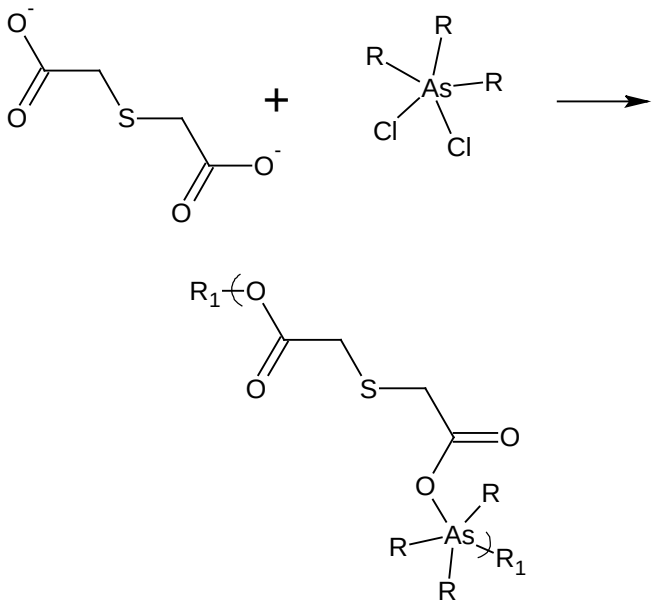


Fig. 2. Synthesis of polymers from reaction of the salt of thiodiglycolic acid with triphenylarsenic dichloride. R_1 is simply chain extension.

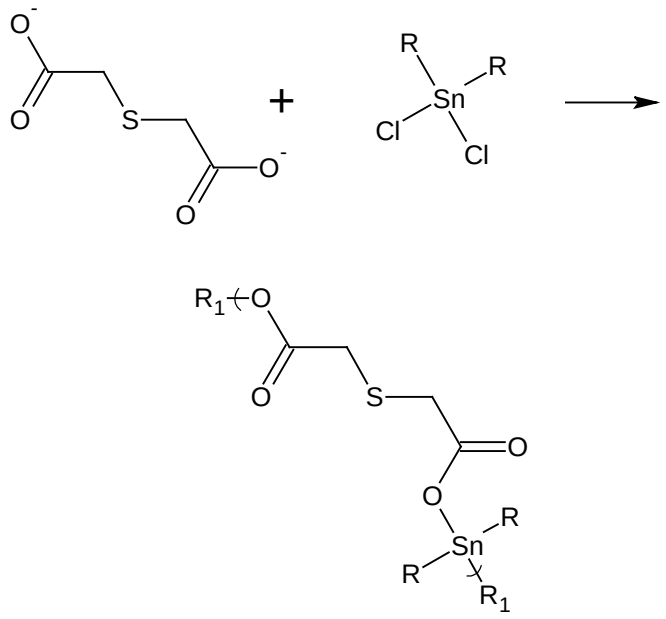
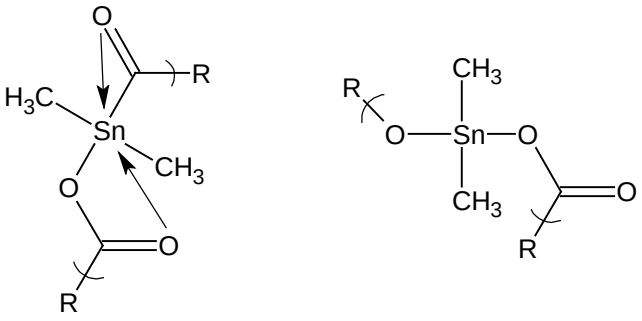


Fig. 3. Synthesis of polymers from reaction of the salt of thiodiglycolic acid with organotin dihalides. R_1 is simply chain extension.



Octahedral, Bridging Tetrahedral, Non-bridging

Fig. 4. Bridging and non-bridging structures about the tin atom.

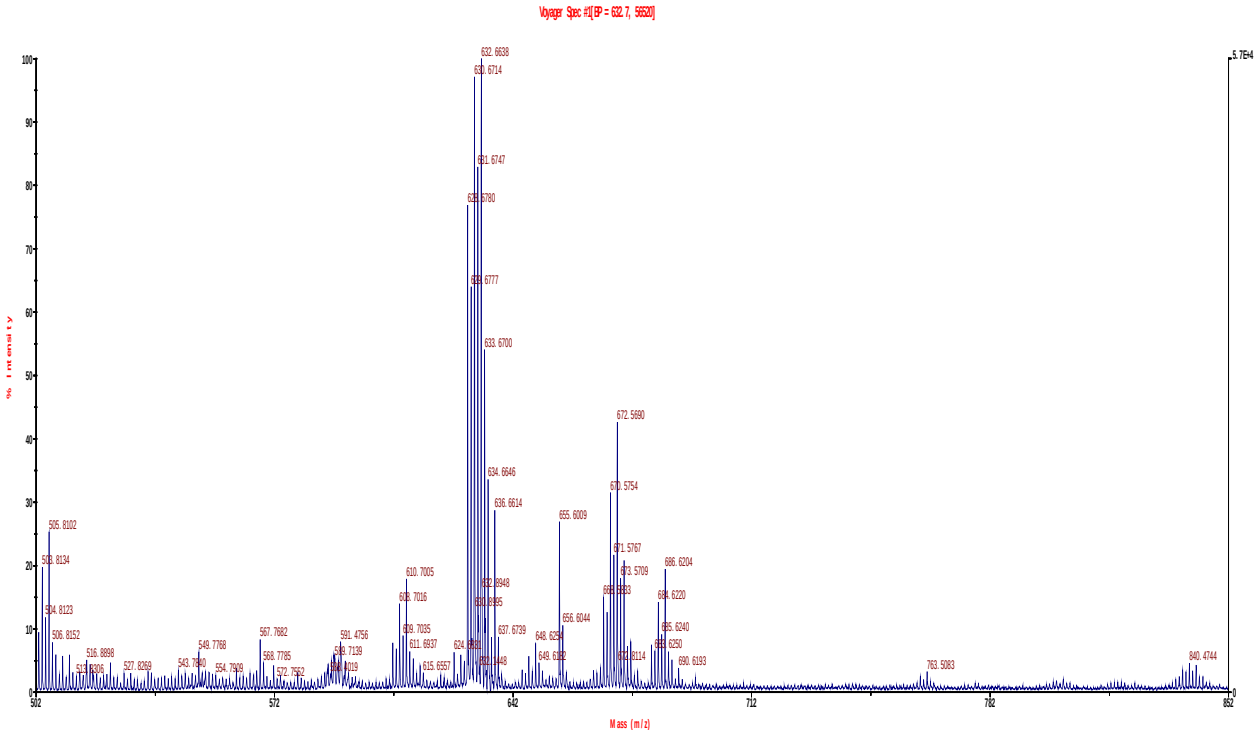


Fig. 5. MALDI MS of the product of dibutyltin dichloride and TA over the range of 445-950.

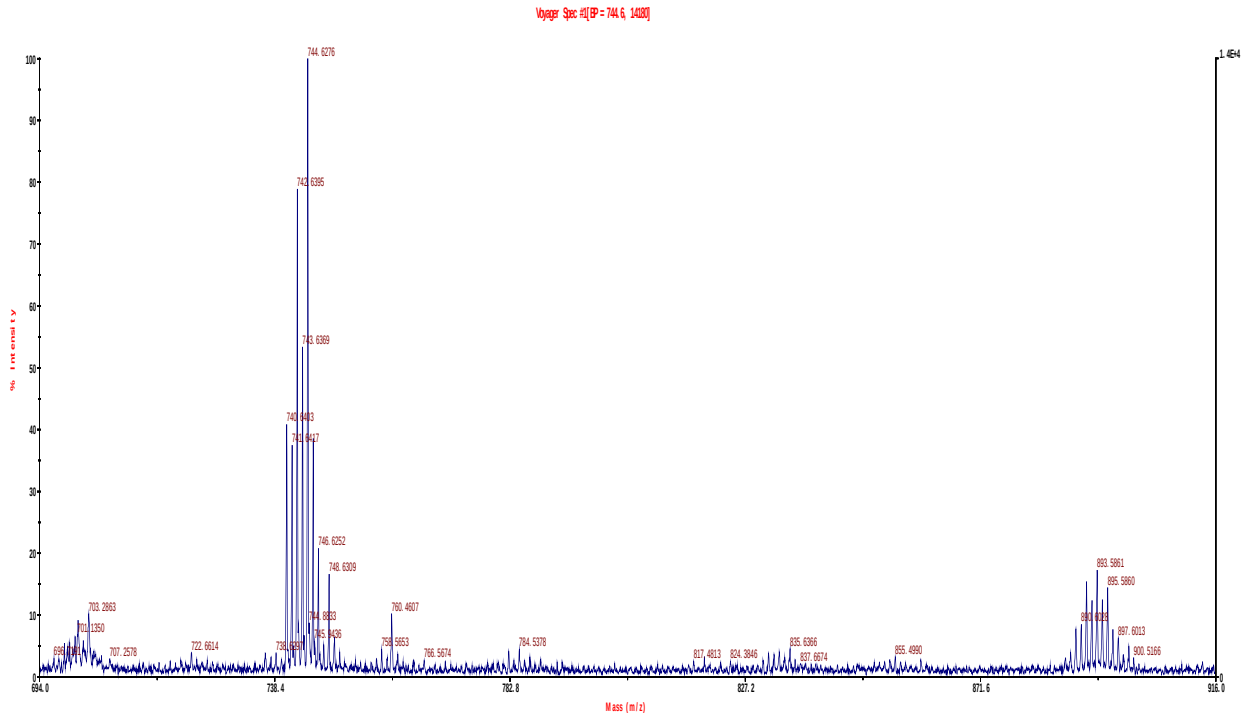


Fig. 6. MALDI MS of the product of dimethyltin dichloride and TA over the approximate range of 700-900.

.

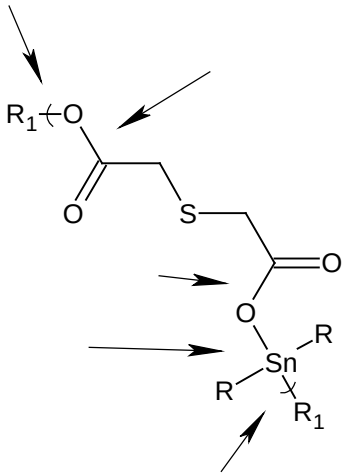


Fig. 7. Preferential locations for bond scission for MALDI MS.

Tables

Table 1. Percentage yields and chain lengths of the products from reaction of thiodiglycolic acid, TA, and organotin dihalides.

Organotin Moiety	% Yield	Molecular Weight	Chain Length
Me_2Sn	82	7.1×10^4	240
Et_2Sn	63	2.9×10^5	890
Bu_2Sn	36	1.3×10^5	340
Cy_2Sn	53	1.1×10^4	25
Oc_2Sn	2	1.0×10^4	20
Ph_2Sn	91	5.0×10^5	1,200

Table 2. Infrared assignments for the monomers and polymer produced from reaction of TA and dibutyltin dichloride.

Assignment	Bu_2SnCl_2	TA	Polymer
CH st	2959,2927, 2872,2858	2943,2910	2951,2925, 2870,2859
C=O non-bridging		1683	1633
CH_3 asy bend	1462		1463
C=O non-bridging		1423	1423
CH_3 sym bend	1380		1380
CH ip bend		1300	1300
CH_2 sym wag		1208	1210
CH_2 asym wag		1190	1190
C-C st	1178		1180
C-C st	1152		1163
Sn-O			1020
CH op bend		915	927
CH_3 rock	878		873

CH st	830	851
C-S st	788	771

Table 3. Infrared assignments for the monomers and polymer produced from reaction of TA and diphenyltin dichloride.

Assignment	Ph_2SnCl_2	TA	Polymer
CH aromatic	3568,3068,3051		3594,3068,3046
CH aliphatic		2943,2910	2961,2930
C=O non-bridging		1683	1636
Sn-Ph st	1480		1480
C=C st	1432,1333		1430,1331
C=O non-bridging		1423	1420
CH ip bend		1300	1305
CH_2 sym wag		1208	1215
CH_2 asym wag		1190	1193
Sn-Ph st	1071		1070
Sn-O st			1021
Ring vib	996		997
CH op bend		915	917
CH st		830	830
C-S st		788	745

CH op bend	729	727
Asy op bend*	691	692

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

Table 4. Presence of bridging and non-bridging associated bands and location.

Organotin Moiety	**Asym. Non-bridging**	**Sym Non-bridging**	**Asym Bridging**	**Sym Bridging**

Me_2Sn	1638(l)	1389(m)	1568(l)	1421(l)
Et_2Sn	1659(l)	1385(m)	1539(l)	1424(l)
Bu_2Sn	1632(l)	1385(m)	1540(l)	1423(l)
Cy_2Sn	1615(l)	1379(m)		1417(s)
Oc_2Sn	1629(l)	1379(m)		
Ph_2Sn	1635(l)	1387)	1565(l)	1430(l)

Where l=large, m=medium, and s=small.

Table 5. Most abundant ion fragment clusters derived from the products of TA and dibutyltin dichloride from 500 to 1100 Da.

Mass (Da)/Assignment	**Mass (Da)/Assignment**

506	U+T-CO	673	U+Bu_2Sn,CO_2,O
530	U+T	687	U+Bu_2Sn,CO_2,O
550	U+T+Na	764	2U
591	U+BuSn,CO	824	2U+T-$2CO_2$
611	U+Bu_2Sn	842	2U+T,Na,-$2CO_2$
632	U+ Bu_2Sn,O	868	2U+T-CO_2
646	U+ Bu_2Sn,CO	1002	2U+ Bu_2Sn

Table 6. Isotopic abundance matches for two ion fragment clusters containing a single tin atom. (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**U+O,Na**		**U+T-CO**	
116	45	418	41	502	44

117	24	419	22	503	20
118	75	420	75	504	76
119	26	421	27	505	26
120	100	422	100	506	100
122	14	424	13	508	14
124	17	426	16	510	18

Table 7. Isotopic abundance matches for two tin-containing ion fragment clusters containing two tin atoms. (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**U+Sn,Na**		**U+Bu_2Sn,Na**	
232	12	835	12	948	12
233	13	836	14	949	12

234	43	837	44	950	43
235	35	838	34	951	33
236	94	839	92	952	80
237	51	840	50	953	51
238	100	841	100	954	100
239	35	842	34	955	32
240	81	843	83	956	81
242	32	845	31	958	30
244	22	847	21	960	18

Table 8. Most abundant ion fragment clusters derived from the products of TA and dibutyltin dichloride from 500 to 1300 Da.

Mass (Da)	**/Assignment**	**Mass (Da)**	**/Assignment**
534	2U-CO_2,Me	834	2U+MeSn,2CO_2
550	2U-CO_2	860	2U+MeSn,2CO_2,Na
592	2U	893	3U

656	2U+T-2CO_2	1029	3U+T-O
700	2U+T-CO_2	1066	3U+Me_2Sn,Na
744	2U+T	1149	4U-CO_2
761	2U+Me_2SnO	1256	4U+T-2CO_2
781	2U+Me_2Sn,O,Na		

Table 9. Isotopic abundance matches for two tin-containing ion fragment clusters containing two tin atoms. (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**2U-CO_2,Me**		**2U+T**	
232	12	529	12	738	12
233	13	530	13	739	14
234	43	531	42	740	48

235	35	532	36	741	32
236	94	533	94	742	89
237	51	534	52	743	50
238	100	535	100	744	100
239	35	536	35	745	34
240	81	537	75	746	62
242	32	539	31	748	28
244	22	541	24	750	22

Table 10. Isotopic abundance matches for one tin-containing ion fragment clusters containing three tin atoms. (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**3U**	
350	14	887	13
351	17	888	15
352	42	889	41
353	41	890	41

354	77	891	79
355	60	892	60
356	100	893	100
357	57	894	60
358	88	895	87
359	37	896	32
360	63	897	58
361	17	898	15
362	34	899	32
363	8	900	6
364	18	901	17

Table 11. Cell line Characteristics and Identification.

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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Table 12. EC_{50} values (micrograms/mL) for the tested cell lines for thiodiglycolic acid (TA) and the tin-containing monomers and polymers. Values given in () are the standard deviations.

Compound	WI-38	NIH/3T3	AsPC-1	PANC-1	PC-3	*MDA	HT-29	MCF-7
Me_2SnCl_2	0.22(.1)	0.43(.1)	0.71(.1)	0.80(.1)	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)
Me_2Sn/TA	1.6(1)	0.12(.1)	>60	>60	>60	>60	>60	>60
Et_2SnCl_2	0.20(.009)	0.46(.038)	0.90(.08)	0.84(.1)	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)
Et_2Sn/TA	0.44(.2)	0.05(.01)	1.8(.9)	4.0(.2)	1.2(.08)	1.7(1)	2.5(1)	2.2(1)
Bu_2SnCl_2	0.20(.05)	0.0035(.005)	>0.15	>0.15	1.4(.11)	1.4(.12)	1.2(.11)	0.70(.06)
Bu_2Sn/TA	0.001(.001)	0.006(.002)	0.056(.01)	0.054(.01)	0.0014	0.0021	0.0017	0.0019

					(.001)	(.001)	(.001)	(.001)
Cy_2SnBr_2	0.22(.04)	0.23(.09)	0.67 (.11)	0.45(.1)	0.48(.1)	0.45(.1)	0.50(.1)	0.59(.1)
Cy_2Sn/TA	0.00011	0.00009	0.016(.06)	0.0015(.001)	0.0034	0.0017	0.0028	0.0025
	(.00008)	(.00001)			(.001)	(.001)	(.001)	(.002)
Ph_2SnCl_2	0.25(.1)	0.66(.16)	0.83(.13)	0.71(.1)	0.82(.1)	0.76(.1)	0.56(.1)	0.59(.1)
Ph_2Sn/TA	0.0033(.001)	.0024(.001)	0.019(.009)	0.14(.09)	0.024(.001)	0.033(.02)	0.027(.01)	0.045(.02)
TA	>60	>60	>60	>60	>60	>60	>60	>60
Cisplatin	0.05(.04)	1.2(.019)	1.4(.15)	0.34(.012)	1.0(.1)	1.0(.1)	2.0(.21)	3.0(.28)

*MDA = MDA MB-231

Table 13. CI_{50} values for the tested cell lines for thiodiglycolic acid and the tin-containing monomers and polymers using WI-38 (top) and NIH 3T3 (bottom) as standards.

Compound	WI-38/ WI-38	WI-38/ NIH/3T3	WI-38/ AsPC-1	WI-38/ PANC-1	WI-38/ PC-3	WI-38/ *MDA	WI-38/ HT-29	WI-38/ MCF-7
Me_2SnCl_2	1	0.51	0.31	0.28	0.43	0.50	0.39	0.33
Me_2Sn/TA	1	13	--	--	--	--	--	--
Et_2SnCl_2	1	0.43	0.22	0.24	0.33	0.31	0.28	0.26
Et_2Sn/TA	1	9	0.2	0.1	0.4	0.3	0.2	0.2
Bu_2SnCl_2	1	1.0	--	--	0.14	0.14	0.17	0.29

Bu_2Sn/TA	1	0.2	0.02	0.02	0.7	0.5	0.6	0.5
Cy_2SnBr_2	1	0.95	0.32	0.49	0.46	0.49	0.44	0.37
Cy_2Sn/TA	1	1.2	0.007	0.007	0.03	0.06	0.04	0.04
Ph_2SnCl_2	1	0.37	0.30	0.35	0.30	0.32	0.45	0.42
Ph_2Sn/TA	1	1.4	0.2	0.2	0.1	0.1	0.1	0.07
Cisplatin	1	0.42	0.36	0.15	0.05	0.05	0.03	0.02

*MDA = MDA MB-231

Compound	NIH/3T3/ WI-38	NIH/3T3/ NIH/3T3	NIH/3T3/ AsPC-1	NIH/3T3/ PANC-1	NIH/3T3/ PC-3	NIH/3T3/ *MDA	NIH/3T3/ HT-29	NIH/3T3/ MCF-7
Me_2SnCl_2	2.0	1	0.61	0.54	0.84	0.98	0.77	0.65
Me_2Sn/TA	0.8	1	--	--	--	--	--	--
Et_2SnCl_2	2.3	1	0.51	0.55	0.75	0.72	0.65	0.60
Et_2Sn/TA	0.1	1	0.02	0.01	0.04	0.03	0.02	0.02
Bu_2SnCl_2	1.0	1	--	--	0.14	0.14	0.17	0.29
Bu_2Sn/TA	6	1	0.1	0.1	4.3	2.9	3.5	3.2
Cy_2SnBr_2	1.0	1	0.34	0.51	0.48	0.51	0.46	0.39
Cy_2Sn/TA	0.8	1	0.006	0.06	0.03	0.05	0.03	0.03
Ph_2SnCl_2	2.6	1	0.80	0.93	0.80	0.87	1.2	1.1
Ph_2Sn/TA	0.7	1	0.1	0.02	0.1	0.07	0.09	0.05
Cisplatin	24.	1	0.86	3.5	1.2	1.2	0.60	0.40

*MDA = MDA MB-231

CHAPTER 8

SYNTHESIS AND STRUCTURAL AND INITIAL CANCER CELL LINE CHARACTERIZATION OF ORGANOTIN POLYESTERS FROM DIPICOLINIC ACID[6]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 12 and 13) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher, Charles E., Paul P. Slawek, Michael R. Roner, Alisa Moric-Johnson, **Lindsey C. Miller**, Jeffrey D. Einkauf, and Floyd Russell

Carraher, Charles E., Paul P. Slawek, Michael R. Roner, Alisa Moric-Johnson, **Lindsey C. Miller**, Jeffrey D. Einkauf, and Floyd Russell (**2016**). "Synthesis and Structural and Initial Cancer Cell Line Characterization of Organotin Polyesters from Dipicolinic Acid." Journal of Inorganic and Organometallic Polymers and Materials 26.6, 1338-350.

[6] Used with permission of Springer, 2017

Synthesis and Structural and Initial Cancer Cell Line Characterization of Organotin Polyesters From Dipicolinic Acid

Charles E. Carraher, Jr.,[a] Paul Slawek,[a] Michael R. Roner,[b] Alisa Moric-Johnson,[b] Lindsey C. Miller[b] , Jeffery, D. Einkauf,[a] Floyd Russell[a]

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 (carraher@fau.edu)

b. University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Dedication

This paper recognizes the many contributions that Professor Dr. Anatoli D. Pomogailo made to the field of polymers and chemistry in general. He is a dear friend and fellow comrade and we will greatly miss him.

Abstract The interfacial polymerization is employed to produce high polymer poly(ester ethers) from the reaction of the salt of dipicolinic acid and various organotin dihalides. They are rapidly synthesized with yield increasing as the length of the organotin alkyl chain increases. Infrared spectroscopy shows the formation of new bands derived from the Sn-O and Sn-O(CO) linkages. It also shows that the products exist as a combination of molecular geometry about the tin atom. MALDI MS shows formation of ion fragment clusters to five and six units in length. The products show good inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines.

Key Words Organotin polyesters, organotin polymers, dipicolinic acid, biological warfare, anthrax, interfacial polymerization, MALDI MS, pancreatic cancer, breast cancer

*Corresponding author: Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; fax 561-297-2759; words approximately 5,000.

1 Introduction

The present research is part of an overall effort to synthesize polymers that can be employed in the inhibition of various unwanted microorganisms, here specifically cancer.

Recently, we focused on the synthesis of metal-containing polymers to inhibit the growth of unwanted materials including bacteria, molds, yeasts, cancers and viruses. This has been

recently reviewed for organotin materials [1]. Much of this effort involves coupling the biologically active organotin moiety with Lewis bases that are known to offer biological activity. Here we describe the synthesis, characterization, and preliminary cancer cell inhibition results of organotin-containing polymers derived from reaction with dipicolinic acid, DPA (Figure 1). DPA is largely known because of its relationship to bacteria belonging to the Bacillus genus, mainly B. anthracis. Much of the interest involves its detection because it is considered a possible biological warfare agent [2]. DPA comprises 5 to 15% dry weight of bacterial spores [2]. It is believed to be responsible for the heat resistance of endospores interacting with DNA through formation of calcium complexes with DPA acting to protect DNA from heat denaturation by inserting itself between nucleobases within the DNA [3].

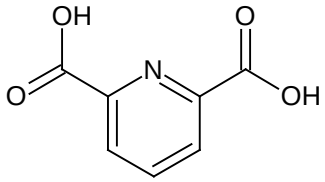


Fig. 1 Structure of dipicolinic acid.

DPA itself is often thought of as being largely biologically inactive though this is not the case. It is known as an enzyme inhibitor [4]. It prevents oxidation of low-density lipoprotein that is involved in the pathogenesis of arteriosclerosis [5]. DPA inhibits platelet aggregation and promotes the synthesis of tissue-type plasminogen activator, t-PA [6]. Thus, DPA is biologically active fitting our typical scenario of combining Lewis acids and bases that are both biologically active.

A number of complexes have been synthesized that exhibit biological activity [7]. Colak and co-workers describe the formation of supramolecular compounds of Mn(II) and Zn(II) through reaction with DPA. These compounds exhibit in vitro antibacterial and antifungal activity. Ilkimen and coworkers described the ability of DPA complexes to inhibit carbonic anhydrase isoenzymes [8]. Yenikaya and coworkers describe the ability of Cu(II) complexes with DPA to inhibit bacterial growth of Staphylococcus aures and Escherichia coli [9].

There are many publications describing the synthesis of polymeric materials from reaction with DPA. These are essentially all describing the formation and properties of metal-organic framework (MOF) and coordination polymers. Yan describes the formation of coordination polymers through coordination of DPA with various lanthanide cations. These products exhibit multi-color luminescence and are useful at detecting Fe^{+3} [10]. Liu reports the formation of similar coordination polymers through reaction of various lanthanide cations with DPA [11]. Additional recent examples are found in references [12-15].

Here we describe the synthesis and characterization of organotin polymers from reaction of the salt of DPA with various organotin dihalides, here dimethyltin dichloride (Figure 2).

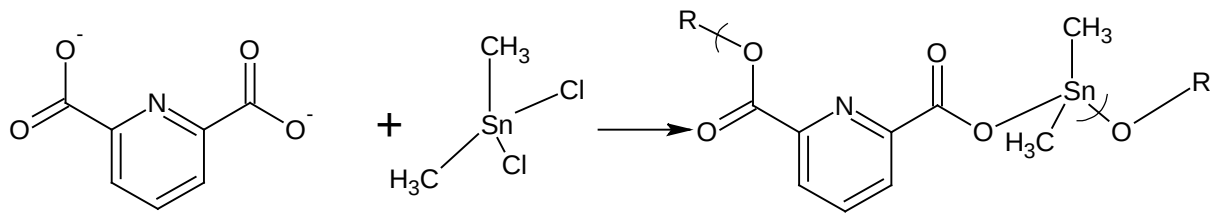


Fig. 2. Reaction scheme between dimethyltin dichloride and the salt of dipicolinic acid forming an organotin polyester

The reaction involves the disalt of dipicolinic acid rather than simply dipicolinic acid itself since the acid is not a sufficient nucleophile to displace the chloride on the organotin dichloride whereas the salt readily displaces the halide forming the ester linkage. The linkage is referred to as an ester linkage because in the naming of these materials, the organometallic is named as a methylene-like moiety or unit. Thus, the created linkage is an ester.

2 Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the dipicolinic acid (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 ^{o}C). Stirring was begun and a hexane solution (30 ml) containing the organotin dihalide (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 hexane to remove unreacted materials and unwanted by-products. The white solid was washed onto a glass petri dish and allowed to dry at room temperature.

Diphenyltin dichloride (1135-99-5), dipicolinic acid (499-83-2) 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.

2.2 Physical Characterization

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ^{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 Da. A graphite matrix was employed. Graphite from a number 2 pencil was marked on the sample holder and sample placed onto the graphite mark.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human pancreas adenocarcinoma cell line (AsPC-1), human pancreas epithelioid duct carcinoma cell line (PANC-1) or mouse embryo-fibroblast (NIH/3T3) cell line. Following a 24 h incubation period, the text compounds were added 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. 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 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.

3 Results and Experimental

3.1Yields and Chain Lengths

Reaction occurs with all of the organotin dihalides. Table 1 contains the product yield, molecular weight and chain length (degree of polymerization, DP) for the products.

Table 1 Product yield, molecular weight and chain length (DP) as a function of organotin moiety for the reaction of organotin dihalides with the salt of dipicolinic acid

Organotin Moiety	% Yield	Mol. Wt. (Daltons)	DP
Me_2Sn	57	3.1×10^5	980
Et_2Sn	84	1.4×10^4	41
Bu_2Sn	99	8.8×10^4	220

Oc_2Sn	100	1.7×10^5	330
Ph_2Sn	90	7.1×10^4	160

Reaction is rapid occurring within 15 seconds stirring time. Yield increases as the length of the organotin alkyl chain increases. Reaction with the salts of dicarboxylic acids occurs within the aqueous layer [16]. Solubility in water of the organotin decreases as the alkyl chain length increases. It is possible that the longer, less water soluble organotin dihalides prevent premature hydrolysis of the tin dihalide allowing for more complete reaction.

Chain length (degree of polymerization, DP) varies from 41 to 980 with the highest chain length being for the dimethyltin product consistent with steric hindrance also playing a role in product chain growth though the lowest chain length is for the diethyltin product which is the next largest alkyl substitutant. The most bulky alky substituent, the octyl moiety, also gives a decent chain length. Thus, while there may be a tendency, it is not strong and additional factors are at play in controlling chain length.

3.2 Infrared Results

Infrared spectral analysis was carried out for all of the samples over the range of 4000-400 cm^{-1}. All band locations are given cm^{-1}.

Infrared spectral analysis is consistent with the proposed structure and with other reported analyses [17-19]. Table 2 contains results for the dibutyltin and diphenyltin polymers. All spectra show bands characteristic of both reactants and a new band for the product assigned to the Sn-O linkage.

Table 2 Assigned peaks for the monomers and associated polymers derived from reaction with dipicolinic acid and dibutyltin dichloride and diphenyltin dichloride

Band Assignment	**Dipicolinic Acid,DPA**	**Bu_2SnCl_2**	**Bu_2Sn/ Polymer**	**Ph_2SnCl_2**	**Ph_2Sn/ Polymer**
CH ip	3108		3108		3094
CH aromatic				3068,3051	3068,3050
CH st	3066		3066		3058
CH_3 asym st		2959	2958		
CH_2 asym st		2926	2928		
CH_3 sym st		2872	2874		
CH_2 sym st		2858	2859		

C=O	1690,1640		1652,1648		1650,1621
Ring st	1574,1565		1587,1572		1567
Sn-Ph st				1480	1480
Ring st-ring C	1457,1465		1457,1465		1488,1480
C=C st				1432	1433
O-H ip wag	1377,1330				
Sn-O asy st			1276		1285
Sn-Ph st				1071	1070
C-C st		1178,1152	1187,1158		
C-O st	1151		1147		1154
Sn-O(CO)st			1031		1024
Ring breathing	996		986	996	997
Skeletal st	889		892		890
CH_3 rock		878	873		
Sn-O sym st			775		771
Sym op bend H's				729	725
Asy op bend ring				691	692
Ring bend	648		649		650
Sn-CH_2 asym st		592	590		
Sn-CH_2 sym st		509	515		
OH op wag	577				
Sn-Ph asym st				442	434

Bands consistent with the formation of the Sn-O bond are found corresponding to the symmetric and asymmetric stretches and a band is found assigned to the stretching for the Sn connected to the oxygen of the carboxylic moiety. Table 3 contains band locations for each of the synthesized polymers.

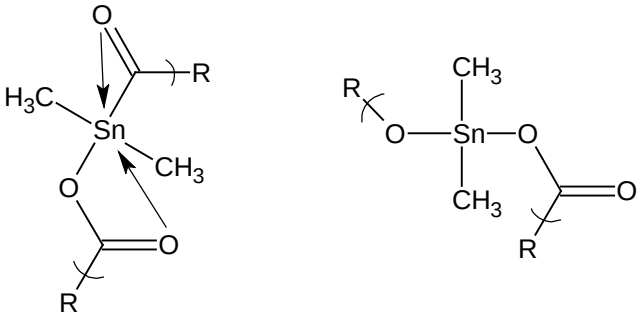
Table 3 Assigned peaks associated with the formation of the Sn-O linkage

Organotin Moiety	Sn-O Asym. St.	Sn-O-(CO) St.	Sn-O Sym. St.

Me_2Sn	1278	1029	788
Et_2Sn	1278	1028	769
Bu_2Sn	1276	1031	775
Oc_2Sn	1279	1029	781
Ph_2Sn	1285	1024	771

The polyesters can exist as bridging or distorted octahedral and non-bridging or distorted tetrahedral about the tin (Fig. 3) [1]. Infrared spectroscopy is the easiest way to determine the structure about the tin. Bridging asymmetric carbonyl absorptions are found around 1570 (all infrared bands are given in cm^{-1}). The bridging symmetric carbonyl band is found around 1410-1435. Non-bridging asymmetric carbonyl bands are found about 1600-1650; and the corresponding symmetric carbonyl band found about 1350-1380.

Table 4 contains results for all of the products with respect to bands associated to bridging/non-bridging. All of the products show a mix of bands consistent with the structures consisting of both bridged and non-bridges geometries about the tin atom.



Octahedral, Bridging Tetrahedral, Non-bridging

Fig. 3. Bridging and non-bridging structures about the tin atom for the product derived from dimethyltin dichloride.

Table 4. Presence of bridging and non-bridging associated bands and location.

Organotin Moiety	**Asym. Non-bridging**	**Sym Non-bridging**	**Asym Bridging**	**Sym Bridging**
Me_2Sn	1614(l)	1359(l)	1571(ml)	1427(l)

Et_2Sn	1615(l)	1358(l)	1572(m)	1425(l)
Bu_2Sn	1614(l)	1358(m)	1572(m)	1423(ml)
Oc_2Sn	1614(ml)	1360(m)	1573(m)	1424(ml)
Ph_2Sn	1621(ml)	1372(l)	1576(l)	1433(l)

Where l=large, m=medium, and s=small.

3.3 MALDI MS

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. Matrix-assisted desorption/ionization was independently introduced in 1981 by Barber and Liu and coworkers [20,21. The addition of the laser as the energy source was introduced by Tanaka, Hillenkamp and coworkers in 1988 [22,23]. The combinations of these concepts allowed the creation of matrix-assisted laser/desorption mass spectroscopy, MALDI MS. We have been using a modification of this technique that allows MALDI MS to be obtained on non-volatile and insoluble products. This approach has been recently reviewed [24-26].

MALDI MS spectra were obtained for the monomers and polymers. Recently we have been employing graphite as the matrix material because it gives good results with few interfering ion fragments produced above 500 mass which is the typical lower mass range employed in our studies [27,28]. Two general MALDI MS modes were employed. These are the reflective and linear mode. 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. Following are results for two of the polymers.

A portion of the MALDI MS for the diphenyltin polymer is given in Figures 4 and 5. Each of the ion fragment clusters above 500 (all ions are given in daltons, Da, or m/e =1) are actually clusters of ions that are produced because of the presence of tin atom(s) within each cluster. Because tin has isotopes, different ion fragments are created that have the same structural formula but vary by the particular tin isotope present. This creates what is often referred to as spectral "fingerprints" characteristic of the natural abundance of these isotopes. The fragments given in the following tables are actually clusters of such ion fragments. Along with the pictorial representations as given in Figures 4 and 5, such distributions can also be presented in table form and compared with known values. Table 5 contains the most abundant ion fragment clusters from Figures 4 and 5. The following abbreviations are utilized to describe the tentative ion fragment cluster assignments- PDA = dipicolinic acid minus two hydrogen atoms; Ph= phenyl moiety, O = oxygen. Additional less abundant ion fragment clusters are also present. Sodium is a common contaminant.

Ion fragments to five units are found. Loss of the organic substitutes on the metal atom often occur and appear only at the site of chain scission (refs 24-26). This is found here.

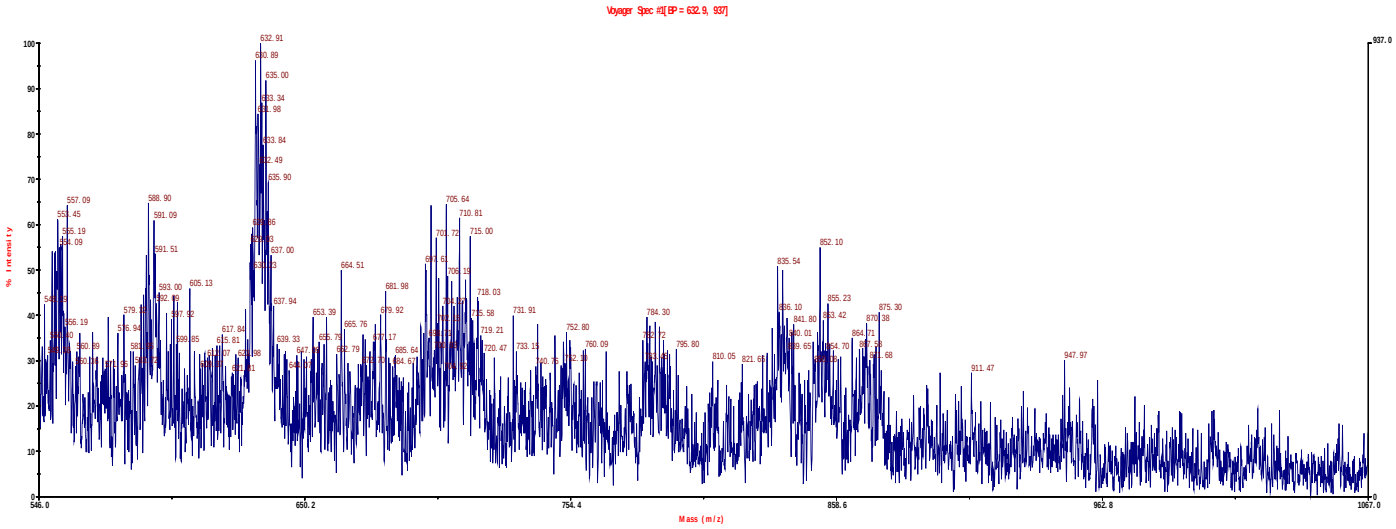


Fig. 4 MALDI MS for the product of diphenyltin dichloride and dipicolinic acid over the approximate mass range of 550 to 1050 Da in the linear mode

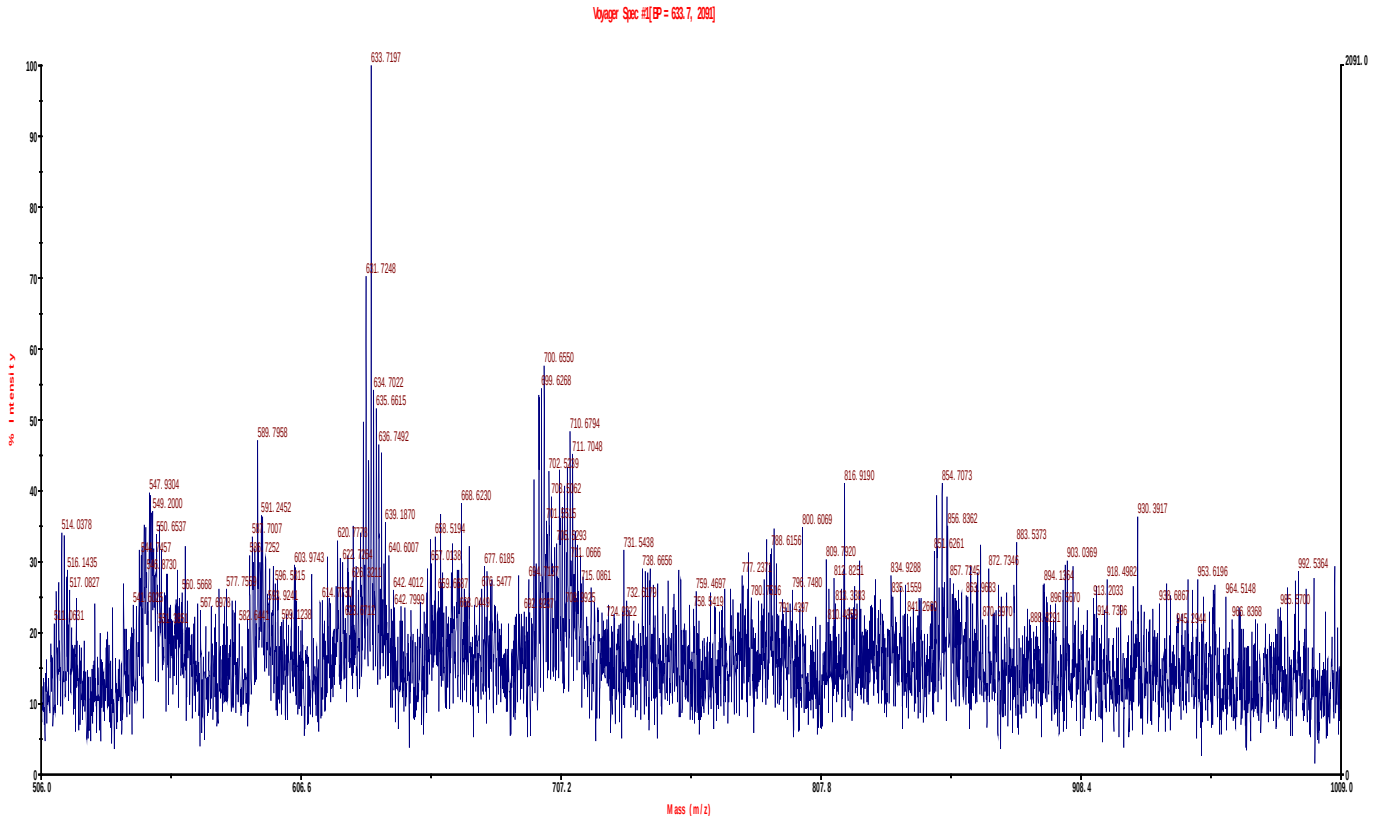


Fig. 5 MALDI MS for the product of diphenyltin dichloride and dipicolinic acid over the approximate mass rante of 500 to 900 Da in the reflective mode

Table 5 Most abundant ion fragment clusters for the product of diphenyltin dichloride and dipicolinic acid over the approximate mass range of 500 to 2300 Da

Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment
553	548	U+PDA,Na-Ph
589	590	U+PDA-O
633	634	U+PDA,Na
706		U+Ph_3Sn
784		U+ Ph_2Sn,O
836		U-CO_2
	855	2U+Na-CO_2
	930	2U+CO_2
948		2U+CO_2,Na
961		2U+DPA-2CO_2
976		2U+DPA,Na-2CO_2
1023		2U+DPA-0
1078	1079	2U+ Ph_2Sn

1114		2U+ Ph_2Sn,CO_2
	1188	2U+ Ph_3Sn,O,Na
1198		2U+ Ph_3Sn,CO_2
1233		2U+ Ph_3Sn,CO_2,O,Na
	1277	3U-CO_2
1295		3U+Na-CO_2
1313		3U
	1322	3U+Na-O
	1355	3U+CO_2
1390	1395	3U+DPA-2CO_2
	1440	3U+DPA-CO_2
1453		3U+DPA-2O
	1522	3U+ Ph_2Sn
	1638	3U+ Ph_3Sn,CO_2
1682		3U+ $Ph_3Sn,2CO_2$
1756		4U
	1852	4U+DPA,Na-2CO_2
	1896	4U+PDA,Na-CO_2
	1964	4U+ Ph_2Sn,O
	1985	4U+ $Ph_2Sn,2O$
	2052	4U+ Ph_3Sn,Na
	2139	5u+ Ph_2Sn,Na
	2198	5U

As noted before, isotopic abundance matches can be employed to support the presence of tin atoms in the isotopic fragment clusters. Tin contains ten isotopes of which seven are considered significant. At higher masses, isotope matches are difficult because of the low intensities of generated ion fragments. Even so, at lower masses such isotope matches are possible. Table 6 contains three isotopic matches for ion fragment clusters containing a single tin atom and Table 7 contains two matches for ion fragment clusters containing two tin atoms. The abundance matches are consistent with the presence of one and two tin atoms within the particular ion fragment clusters.

Table 6 Isotopic abundance matches for two ion fragment clusters containing a single tin atom (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**U+PDA,Na-Ph**		**U+PDA-O**		**U+PDA,Na**	
116	45	544	46	586	45	629	48

117	24	545	26	587	24	630	23
118	75	546	75	588	70	631	73
119	26	547	28	589	27	632	30
120	100	548	100	590	100	633	100
122	14	550	22	592	24	635	23
124	17	552	24	594	26	637	25

Table 7 Isotopic abundance matches for two tin-containing ion fragment clusters containing two tin atoms (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**2U+Na-CO_2**		**2U+CO_2**	
232	12	849	14	924	13
233	13	850	15	925	15
234	43	851	44	926	44
235	35	852	36	927	37
236	94	853	94	928	89
237	51	854	51	929	51
238	100	855	100	930	100
239	35	856	33	931	35
240	81	857	89	932	79
242	32	859	34	934	34
244	22	861	24	936	24

The isotopic matches are in reasonable agreement with the standard and consistent with the presence of one and two tin atoms in the fragment clusters. The “mildness” of MALDI MS is shown by the lack of fragmentation of the DPA ring moiety.

Figures 6 and 7 and Table 8 contains results for the product of dibutyltin dichloride and DPA.

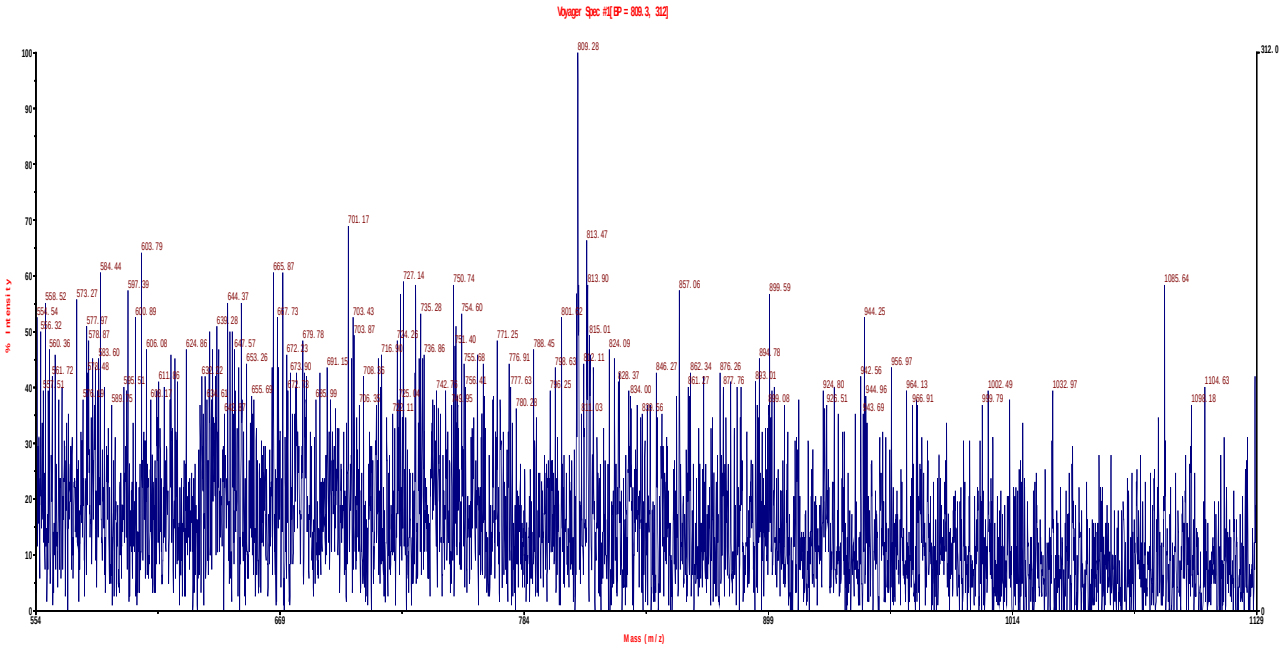


Fig. 6 MALDI MS for the product of dibutyltin dichloride and dipicolinic acid over the approximate mass range of 500 to 1100 Da in the linear mode

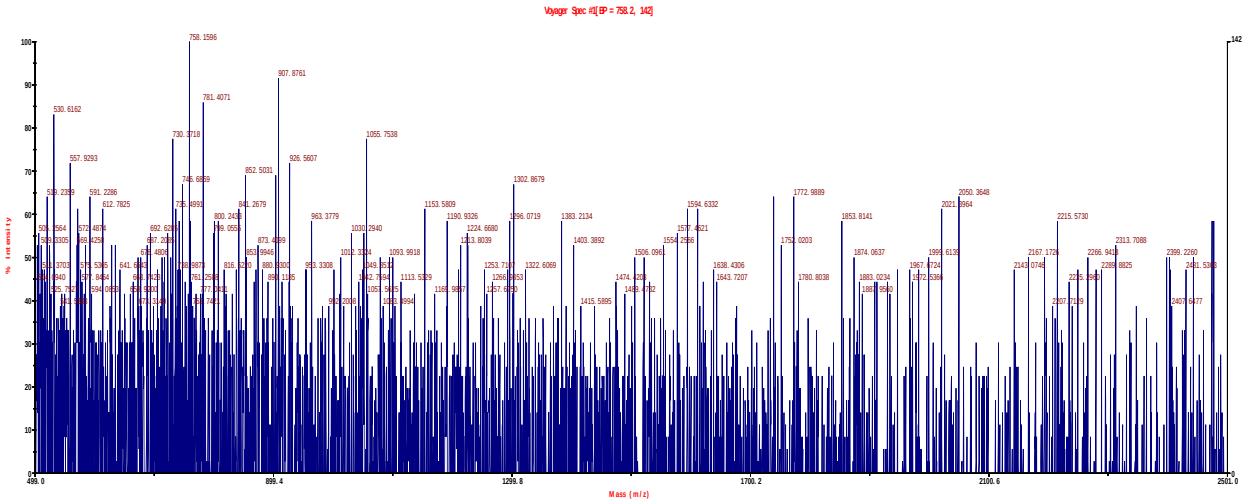


Fig. 7 MALDI MS for the product of dibutyltin dichloride and dipicolinic acid over the approximate mass rante of 500 to 2500 Da in the reflective mode

Table 8 MALDI MS results for the polymer derived from dibutyltin dichloride and dipicolinic acid

Mass,Da/	Mass,Da/	(Tentative) Assignment

Linear	Reflective	
	562	U+PA
	571	U+PA,Na-O
666		U+Bu_2Sn,2O
693	693	U+ Bu_2Sn,20,Na
701		U+ Bu_2Sn,CO_2Na
756	758	2U-CO_2
771		2U+Na-CO_2
781	781	2U-O
801	800	2U
857		2U+CO_2,Na
	914	2U+PA-CO_2
	927	2U+PA-20
964	963	2U+PA
1033	1030	2U+ Bu_2Sn
1055	1056	2U+ Bu_2Sn,Na
1085		2U+ Bu_2Sn,20,Na
1098		2U+ Bu_2Sn,C0_2,Na
1026		2U+ Bu_2Sn,2CO_2
	1154	3U-CO_2
1190	1190	3U
1208		3U+O
	1224	3U+Na
1301	1302	3U+PA-2CO_2
1386	1383	3U+PA,Na
1451		3U+ Bu_2Sn,Na
1499	1498	3U+ Bu_2Sn,CO_2,Na
1544	1542	3U+ Bu_2Sn,2CO_2
	1577	4U-O
	1595	4U
1624		4U+Na
	1638	4U+CO_2
1667		4U+CO_2,Na
1740		4U+PA-O
	1752	4U+PA
	1773	4U+PA.Na-O
	1853	4U+ Bu_2Sn,O
1885	1883	4U+ Bu_2Sn,CO_2,O
1970	1968	5U-O
	2021	5U+Na
	2050	5U+CO_2,Na
2090		5U+PA,Na-CO_2
	2167	5U+PA

2248		5U+ Bu_2Sn,O
	2314	5U+ Bu_2Sn,CO_2,O,Na
2385		6U-O
	2399	6U
2440		6U+CO_2

Ion fragment to 6 units are found. As noted before, tin has a number of isotopes that allow isotopic abundance matching to occur. Table 9 contains isotopic abundance matches for two ion fragment clusters containing one tin atom and Table 10 contains isotopic abundance matches for two clusters containing two tin atoms. The results are consistent with the clusters containing tin atoms.

Table 9 Isotopic abundance matches for two ion fragment clusters from the MALDI MS of the product of dibutyltin dichloride and dipicolinic acid containing a single tin atom (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**U+PA,Na-O**		**U+T-CO**	
116	45	567	42	580	42
117	24	568	22	581	24
118	75	569	72	582	72
119	26	570	23	583	26
120	100	571	100	584	100
122	14	573	15	586	15
124	17	575	18	588	18

Table 10 Isotopic abundance matches for two tin-containing ion fragment clusters from the MALDI MS of the product of dibutyltin dichloride and dipicolinic acid containing two tin atoms (Only ion fragments >5% relative abundance are reported.)

Known for Sn		**2U+Na-CO_2**		**2U+PA-CO_2**	
232	12	765	14	908	13
233	13	766	15	909	14
234	43	767	41	910	42
235	35	768	32	911	31

236	94	769	93	912	90
237	51	770	51	913	47
238	100	771	100	914	100
239	35	772	34	915	33
240	81	773	82	916	83
242	32	775	28	918	29
244	22	777	22	920	20

As in other studies, chain scission occurs at the heteroatoms as shown in Figure 8.

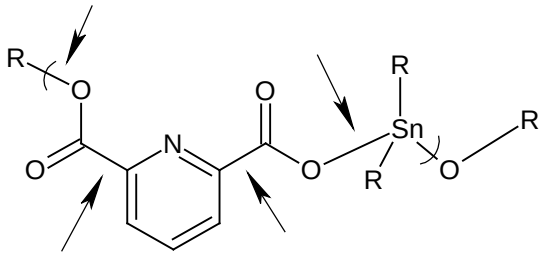


Fig 8 Locations of preferred bond scission

3.4 Proton NMR

Proton NMR was carried out on the monomers and polymers in d-6 DMSO. All bands are given ppm. DPA shows two bands at 8.77 (ortho protons) and 8.48 (meta and para protons) assigned to the ring protons and 12.2 for the hydroxyl proton which is absent in the polymers. Dibutyltin dichloride exhibits bands at 1.5 (closest to the tin), 1.6, 1.3 and 0.85 (furthest from the tin) derived from the n-butyl chain. The DPA/Bu_2Sn polymer shows bands at 8.7 and 8.3 from the DPA and 1,4, 1,3, 1,1 and 0.85 from the butyl chain.

Diphenyltin dichloride shows bands at 7.9 (ortho), 7.4 and 7.3 (meta and para). The corresponding polymer exhibits bands at 8.5 and 8.2 from DPH and from the diphenyltin moiety 7.7, 7.4, and 7.3. Dimethyltin dichloride shows a band at 1.3. The dimethyltin polymer has bands from DPH at 8.5 and 8.3 and from the methyltin moiety at 1.3.

Thus, proton nmr shows bands derived from both reactants within the products. There are only mild shifts between the monomers and polymer. For the dibutyltin product there are mild changes for the two methylenes that are closest to the tin atom. Because of the poor solubility of the polymer, additional data is not confidently available.

3.5 Cancer Cell Lines

Much of our recent activity has focused on the synthesis and preliminary cancer cell line testing of various organotin-containing polymers. Table 11 contains the cell lines employed in the current study.

Table 11 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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

While different measures have been employed in the evaluation of cell line results, the most widely employed involve the concentration, dose, needed to reduce the growth of the particular cell line. Here we will employ the term effective concentration, EC. 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 here is given the symbol EC_{50}. Table 12 contains the EC_{50} values found for the monomers and polymers and include values for cisplatin as a standard.

In general, as the organotin dihalide is varied for a particular Lewis base, the polymer containing the dibutyltin moiety shows the greatest ability to curtail cancer cell line growth followed by those containing the diphenyltin moiety [1]. The next greatest activities were by dipropyltin, diethyltin, and dimethyltin-containing polymers. The lowest activity was generally found for long chain alkyl groups such as dioctyltin. This activity was found for a variety of cancers [1]. Even so, for the current polymers there is no clear trend with the lowest and highest EC_{50} values varying dependent on the particular cancer cell line. For instance, for the PANC-1 pancreatic cancer cell line, the highest EC_{50} value (least toxic) is found for the diethyltin polymer while the lowest EC_{50} values is found for the dimethyltin product. By comparison, for the PC-3 prostate cancer cell line, the highest concentration of polymer, least toxic, is found for the dibutyltin product while the lowest EC_{50} is found for the dioctyltin polymer. In all cases the general toxicity range is relatively small. Thus, there exists no clear general trend with respect to the nature of the organotin moiety.

Table 12 EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values Given in ( ) are Standard Deviations for Each Set of Measurements

Sample	3T3	*WI-38*	*PANC-1*	*AsPC-1*
Me_2SnCl_2	0.43 (.1)	0.22(.1)	0.80(.1)	0.71(.1)
Me_2Sn/DPA	0.67(.09)	0.71(.1)	0.61(.006)	0.60(.09)
Et_2SnCl_2	0.46(.1)	0.20(.1)	0.48(.1)	0.90(.1)
Et_2Sn/DPA	0.72(.09)	0.72(.1)	0.71(.1)	0.69(.09)
Bu_2SnCl_2	0.20 (.05)	0.20(.05)	>15	>15
Bu_2Sn/DPA	0.71(.09)	0.63(.1)	0.69(.1)	0.66(.09)
Oc_2SnCl_2	0.56(.1)	0.30(.1)	0.85(.1)	0.85(.1)
Oc_2Sn/DPA	0.73(.09)	0.68(.1)	0.62(.1)	0.63(.09)
Ph_2SnCl_2	0.66(.1)	0.25(.1)	0.71(.1)	0.83(.1)
Ph_2Sn/DPA	0.63(.09)	0.62(.1)	0.64(.1)	0.67(.09)
Dipicolinic acid	>30	>30	>30	>30
Cisplatin	15(10)	1,200(19)	1,400(150)	340(12)

Sample	*PC-3*	*MDA-MB-231*	*HT-29*	*MCF-7*
Me_2SnCl_2	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)
Me_2Sn/DPA	0.62(.06)	0.62(.08)	0.62(.1)	0.61(.1)
Et_2SnCl_2	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)
Et_2Sn/DPA	0.67(.06)	0.60(.08)	0.64(.1)	0.63(.1)
Bu_2SnCl_2	1.4(1.1)	1.4(1.3)	1.2(.1)	0.70(.06)
Bu_2Sn/DPA	0.70(.09)	0.63(.1)	0.67(.1)	0.67(.09)
Oc_2SnCl_2	0.55(.1)	0.65(.1)	0.65(.1)	0.70(.1)
Oc_2Sn/DPA	0.60(.06)	0.71(.08)	0.63(.1)	0.62(.1)
Ph_2SnCl_2	0.82(.1)	0.76(.1)	0.56(.1)	0.68(.1)
Ph_2Sn/DPA	0.67(.06)	0.63(.08)	0.68(.1)	0.66(.1)
Dipicolinic acid	>30	>30	>30	>30
Cisplatin	1.00(0.10)	3.00(0.28)	2.00(0.21)	1.00(0.1)

There are several general observations from the data presented in Table 12. First, the polymers have a much greater toxicity towards the cancer cell lines compared with DPA, which can be considered nontoxic over the studied range. Second, the polymers generally have a greater toxicity than cisplatin towards the tested cell lines showing EC_{50} values less than cisplatin. Third, the general toxicity of the polymers is similar to that for the organotin monomer. Based on the amount of tin in the materials, the polymers exhibit lower EC_{50} values compared with the organotin monomers. Thus, the combination of the organotin moiety with the Lewis base offers some enhanced ability to inhibit cancer cell growth.

Following focuses on toxicity with respect to particular cancer cell lines. 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 polymers we found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure [1,29-32].

In these studies polymers containing a Lewis base that possesses the O-Phenylene moiety, such as hydroquinone and hydroquinone derivatives [33] and diethylstilbestrol [34], exhibit a relatively 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 DPA in the present study, that do not contain this structural moiety showed little difference between the ability to inhibit the two cell lines [1]. Here, there is little difference between the ability to inhibit the two breast cancer cell lines with the various organotin polymers.

One of our recent focuses regards pancreatic cancer. Pancreatic cancer afflicts close to 32,000 individuals each year in the United States and 168,000 worldwide, and nearly all patients die from the ravages of their disease within 3 to 6 months after detection. 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). Treatment of pancreatic cancer is rarely successful as this disease typically metastasizes prior to detection. There is no chemotherapy for metastasized pancreatic cancer. We recently described the ability of a number of organotin polymers to inhibit pancreatic cancer [1,35,36]. Because pancreatic cancer does not have a generally accepted "cure" one of our current focuses is on the synthesis of materials that might be successful in combating pancreatic cancer. 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. Both are human cell lines and are widely employed in testing for inhibition of pancreatic cancer. For the current study with respect to inhibition of the two pancreatic cancer cell lines, all of the polymers offer decent inhibition of both cell lines and being similar for both cell lines is consistent with the idea that the polymers might offer broad-spectra inhibition of other pancreatic cancer cell lines.

The PC-3 results are of interest because this particular prostate cell line is viewed as one of the most resistant of the prostate cancer cell lines [1, 37]. As in the case of the other cell lines, all of the polymers offer decent inhibition of the PC-3 cell line.

As noted before, the there is no overall trend with respect to the nature of the organotin moiety and the toxicities are similar allowing use of the dibutyltin polymers to be the considered for the treatment of all of the cell lines. The possible use of dibutyltin is positive based on the following. First, it is the least expensive of the organotin halides and available in the ton and greater quantities. Second, it is the least toxic to humans of the lower alkyltins. Third, commercially it is the most widely employed organotin moiety. Fourth, is it known to naturally degrade to simple tin oxide offering a low toxic form of degradation product.

Another measure of the potential use of compounds 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, we will simply employ the term chemotherapeutic index, CI, so that the CI_{50} is then the ratio of the EC_{50} for the NIH/3T3 or WI-38 cells divided by the EC_{50} for the particular test cell. These results are shown employing WI-38 and NIH/3T3 cells as the standards in Table 13. 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. In the present case, while there are several instances where the ratio is greater than one, none of the polymers shows values greater than two.

Table 13 CI_{50} results for values calculated from data given in Table 2

Sample	EC_{50} *3T3*/ EC_{50}*3T3*	EC_{50} *3T3*/EC_{50} WI-38	EC_{50} 3T3/ EC_{50}*PANC*-1	EC_{50} *3T3*/ EC_{50}*ASPc-1*
Me_2SnCl_2	1	2	0.54	0.61
Me_2Sn/DPA	1	0.94	1.1	1.1
Et_2SnCl_2	1	1.2	1.0	1.0
Et_2Sn/DPA	1	1.0	1.0	1.1
Bu_2SnCl_2	1	1		
Bu_2Sn/DPA	1	1.1	1.0	1.1
Oc_2SnCl_2	1	1.9	0.66	0.66
Oc_2Sn/DPA	1	1.1	1.2	1.2
Ph_2SnCl_2	1	2.6	0.92	0.80
Ph_2Sn/DPA	1	1.0	0.85	0.94
Cisplatin	1	60	8.8	2.1

Sample	EC_{50} *3T3*/ EC_{50}*PC-1*	EC_{50} *3T3*/ EC_{50}*MDA*	EC_{50} *3T3*/ EC_{50}*HT-29*	EC_{50} *3T3*/ EC_{50}*MCF-7*
Me_2SnCl_2	0.84	0.98	0.77	0.65
Me_2Sn/DPA	1.1	1.1	1.1	1.1
Et_2SnCl_2	1.1	1.1	0.80	0.86
Et_2Sn/DPA	1.1	1.2	1.2	1.1

Bu_2SnCl_2	0.14	0.14	0.17	0.29
Bu_2Sn/DPA	1.0	1.1	1.1	1.2
Oc_2SnCl_2	1.0	0.86	0.86	0.76
Oc_2Sn/DPA	1.2	1.0	1.2	1.2
Ph_2SnCl_2	0.80	0.87	1.2	0.98
Ph_2Sn/ DPA	0.95	1.0	0.94	0.95
Cisplatin	3.0	3.0	1.5	1

Sample	EC_{50} *WI-38*/ EC_{50}*3T3*	EC_{50} *WI-38*/ EC_{50}*WI-38*	EC_{50} *WI-38*/ EC_{50}*PNC-1*	EC_{50} *WI-38*/ EC_{50}*AsPC-1*
Me_2SnCl_2	0.51	1	0.28	0.31
Me_2Sn/DPA	1.1	1	1.1	1.2
Et_2SnCl_2	0.83	1	0.83	0.81
Et_2Sn/DPA	1.0	1	1.0	0.80
Bu_2SnCl_2	1	1		
Bu_2Sn/DPA	0.89	1	0.91	0.95
Oc_2SnCl_2	0.55	1	0.35	0.35
Oc_2Sn/DPA	0.93	1	1.1	1.1
Ph_2SnCl_2	0.38	1	0.35	0.31
Ph_2Sn/DPA	0.98	1	0.97	0.93
Cisplatin	0.02	1	0.15	0.04

Sample	EC_{50} *WI-38*/ EC_{50}*PC-3*	EC_{50} *WI-38*/ EC_{50}*MDA*	EC_{50} *WI-38/* EC_{50}*HT-29*	EC_{50} *WI-38*/ EC_{50}*MCF-7*
Me_2SnCl_2	0.43	0.50	0.39	0.39
Me_2Sn/DPA	1.1	1.1	1.2	1.3
Et_2SnCl_2	0.91	0.91	0.67	0.71
Et_2Sn/DPA	1.2	1.2	1.1	1.1
Bu_2SnCl_2	0.14	0.14	0.17	0.29
Bu_2Sn/DPA	0.90	1.0	0.94	0.94
Oc_2SnCl_2	0.55	0.46	0.46	0.43
Oc_2Sn/DPA	1.1	0.96	1.1	1.1
Ph_2SnCl_2	0.30	0.33	0.45	0.37
Ph_2Sn/DPA	0.93	0.98	0.91	0.94
Cisplatin	0.05	0.05	0.025	0.02

Based on EC_{50} values the polymers show good ability to inhibit the cancer cell lines including two pancreatic cancer cell lines. But, based on CI_{50} values none of the polymers show an outstanding ability to inhibit the tested cancer cell lines. There is not agreement as to which measure, EC_{50} or CI_{50} values, is the best measure of the ability of tested materials to inhibit cancer growth so results employing both measures are presented.

A second study is superimposed onto the current study. This second study involves comparing results from the two most frequently used cell lines as standards involved in the evaluation of the effectiveness of compounds through calculating CI values. These two cell lines are the NIH/3T3 and WI-38 cell lines.

NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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. While NIH/3T3 cells are often favored because of ease of handling aided by an infinite life span, results from WI-38 cells are given greater importance when there is a difference [1,38].

To see if the more easily handled NIH/3T3 cells can be used in place of the WI-38 cell one can simply observe the ratio of EC_{50} for the WI-38 values divided by the EC_{50} for the NIH/3T3. If there is no difference the ratio should be about 1. In the present case, the values range from 1.1 to 0.89 so there is a not a significant difference. Thus, there is little difference between the values obtained using the WI-38 and NIH/3T3 as the control cells and either can be employed as the standard.

4. **Summary**

High polymer poly(ester ethers) are rapidly (15 seconds) formed from the interfacial polycondensation of the salt of dipicolinic acid with various organotin dihalides employing commercially available reactants allowing their ready production in gram to ton quantities. Yield increases (from 57% for the dimethyltin to 100% for the dioctyltin product) as the length of the organotin alkyl chain increases. Infrared spectroscopy shows the formation of new bands derived from the Sn-O and Sn-O(CO) linkages. It also shows that the products exist as a combination of molecular geometries of distorted octahedral and tetrahedral about the tin atom. MALDI MS shows formation of ion fragment clusters to five and six units in length with chain session occurring at the hetro-atom linkages. The dipicolinic ring moiety remains intact. The products show good inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines. The EC_{50} values for the polymers are generally less than that found for cisplatin.

References

1. C. Carraher, M. Roner, J. Organometallic Chemistry, 751, 67-82 (2014)
2. A. Sengupta, C. Shende, S. Farquharson, F. Inscore, Int. J. Spectro., 2012, 5, 2012.
3. T. Sliemandagger, W. Nicholson, App. Env. Microbioloogy, 67, 1274, 2001.
4. M. Kirillova, M. Da Silva, A. Kirilov, J. De Silva, A. Pombeiro, Inorg. Chim, Acta, 360, 506 (2007).
5. Z. Vargova, V. Zeleoak, I. Cisaova, K. Gyoryova, Thermochim. Acta., 423, 149 (2004).
6.T. Ohsugi, H. Sumi, J. Food Biochem., 35, 370 (2011).
7.A. Colak, F. Colak, O. Yesilel, O. Buyukgungor, J. Coord. Chem., 62, 1650, 2009).
8. H. Ilkimen, C. Yenikaya, M. Sari, M. Bulbul, E. Tunca, Y. Suzen, Polyhedron, 61,56 (2013).
9. C. Yenijaya, N., Buhyukkidan, M. Sari, R. Keslis, H. Ilkimen, M. Bulbul, Orhan, Buyukgungor, J. Coord. Chem., 64, 3353 (2011).
10. H. Weng, B. Yan, Inorg. Chem. Communications, 63, 11 (2016).
11. R-Z. Du, Y-Y. Wang, Y-Y. Xie, H-T. Li, T-F. Liu, J. Mol. Structure, 1108, 96 (2016).
12. M. Ghadermazi, Z. Derikvad, A. Zahedi, A. Shokrollahi, S. Nasin, A. Rudbari, Monatschfte Chemie, 146, 1257 (2015).
13. G. Kumar, G. Kuman, R. Gupta, Inorg. Chim. Acta, 425, 260 (2015).
14. S. Sharif, O. Sabin, B. Khan, I. Ullah Khan, J. Coord. Chem., 68, 2725 (2015).
15. H. Li, Syn. React. Inorg., Metal-Org., Nano-Metal Chem., 45, 1664 (2015.

16. C. Carraher, J. Inorg. Organometal. Polym. Mat. 15, 121 (2005).
17. C. Carraher, M. Roner , R. Lambert, L. Arroyo, L. Miller , J. Inorg. Organomet. Polym., 25, 1414, 2015.
18. C. Carraher, N. T. C. Truong, M. Roner, A. Moric-Johnson, N. Sookdeo, N. Trang, Inorg. Chim. Acta, 423 (B), 2014.
19. C. Carraher, A. Morrison, M. Roner, A. Moric, N. Trang. J. Inorg. Organomet. Polym., 24, 182, 2014.
20. Barber, M.; Bordoli, R. S.; Sedwick, R. D.; Tyler, A. N.; Nature, **1981**,293, 270.
21.Liu, L. K.; Busch, K. L.; Cooks, R. G. Analyt. Chem., **1981**, 53, 109.
22. Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y.; Yoshida, T.; Rapid Commun. Mass. Spectrom., **1988**, 2, 151.
23. Karas, M.; Hillenkamp, F. Anal. Chem., **1988**, 60, 2299.
24. Carraher C.; Barot G.; Battin A. J Polym Mater. **2009**, 26, 17.
25. Carraher C.; Sabir T.; Carraher C. L. J Polym Mater. **2006**, 23, 143.
26. Carraher, C.; Sabir, T.; Carraher, C. L., *Inorganic Organometallic Macromolecules*, Springer, NY, 2008, pp. 329.
27. C. Carraher, M. Roner, C. L. Carraher, R. Crichton, K. Black J. Macromol. Sci., A., 52, 867-886, 2015.
28. C. Carraher, V. Suresh, M. Roner , J. Polym. Mater., 32, 151-168, 2015.
29. Carraher, C.; Roner, M.; Shahi, K.; Barot, G. Materials 2011, 4, 801.
30. Carraher, C.; Roner, M.; Ayhoub, M.; Pham, N.; Moric-Johnson, A. Polym. Mater. Polym. Biomat. 2015, 64, 311.
31. C. Carraher, M. Roner, K, Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin,. N. Trang,. N. Sookdeo, Z. Islam, Internat. J. Poly. Mater. , 64, 800-814, 2015.
32. C. Carraher, M. Roner, K. Shahi, G. Barot, Materials 4, 801, 2011.
33. G. Barot, M. Roner, Y. Naoshima, K, Nago, K. Shahi, C. Carraher, J. Inorg. Organometal. Polym., 19, 12, 2009.
34. C. Carraher, M. Roner, K. Shahi, Y. Ashida, G. Barot, J. Inorg. Organometal. Polym., 18, 180, 2008.
35. C. Carraher, M. Ayoub, M. Roner, A. Moric, N. T. Trang, JCAMS, 1, 65, 2013.
36. C. Carraher, M. Roner, K. Shahi, A. Battin, G. Barot, T. Arnold, J. Polym. Mater., 31, 1,2014.
37. C. Carraher, M. Roner, K. Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin, N. Trang, M. Alhuniti, J. Inorg. Organometal. Polym. , 25, 386, 2015.
38. C. Carraher, M. Roner, M. Ayoub, R. Crichton, A. Moric-Johnson, L. Miller, K. Black, J. Macromol. Sci., A, 53, 328-334, 2016.

CHAPTER 9

SYNTHESIS AND PRELIMINARY CANCER CELL LINE RESULTS FOR THE PRODUCT OF ORGANOTIN DIHALIDES AND ALPHA-CYANO-4-HYDROXYCINNAMIC ACID[7]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 11 and 12) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Roner, M.R.; Crichton, R.; Moric-Johnson, A.; **Miller, L.;** Black, K. and Russell, F.

Carraher Jr.,C.E.; Roner, M.R.; Crichton, R.; Moric-Johnson, A.; **Miller, L.;** Black, K. and Russell, F. **2016**. Synthesis and Preliminary Cancer Cell Line Results for the Product of Organotin Dihalides and Alpha-Cyano-4-Hydroxycinnamic Acid. J. Inorg. Organomet. Polym., Online first:DOI 10.1007/s10904-016-0363-1.

[7] Used with permission of Springer, 2017

Synthesis and Preliminary Cancer Cell Line Results for the Product of Organotin Dihalides and Alpha-Cyano-4-Hydroxycinnamic Acid

Charles E. Carraher, Jr.,[a] Michael R. Roner,[b] Ryan Chrichton,[a] Alisa Moric-Johnson,[b] Lindsey Miller,[b] Kendra Black,[a] **Floyd Russell,[a]**

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 and Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410 (carraher@fau.edu)

b. University of Texas Arlington, Department of Biology, Arlington, TX 76010 (roner@uta.edu)

Dedication

This paper recognizes the many contributions that Professor Dr. Anatoli D. Pomogailo made to the field of polymers and chemistry in general. He is a dear friend and fellow comrade and will greatly be missed.

Abstract High polymer poly(ester ethers) are rapidly synthesized in good yield employing the interfacial polycondensation reaction system. Infrared spectroscopy shows the formation of new bands derived from the Sn-O and Sn-O(CO) linkages. It also shows that the products exist as alternating Sn-O and Sn-O(CO) linkages. MALDI MS shows formation of ion fragment clusters

several units in length. The products show reasonable inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines.

Keywords Tin-containing polymers, pancreatic cancer, inhibition of cancer cell lines, interfacial polymerization, MALDI MS, organotin poly(esters ethers)

*Corresponding author: Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; fax 561-297-2759; words approximately 5,000.

1 Introduction

Part of our recent emphasis involves synthesis of metal-containing polymers to fight cancer and to broaden our knowledge of such materials. A recent focus was on organotin-containing polymers. This was recently reviewed [1].

One guiding feature in our effort to suppress various unwanted microbes is coupling the metal-containing moiety, here organotin which is known to have biological activity, with Lewis bases that also offer some biological activity hoping for a synergetic effect from this coupling. Further, it is hoped this coupling results in materials that inhibit the growth or formation of these unwanted microbes at several sites discouraging production of resistant strains.

Alpha-cyano-4-hydroxycinnamic acid, CHA (Figure 1), is known to be biologically active. It impairs glucose oxidation through inhibiting pyruvate transport across the mitochondrial membrane [2]. Related to this, CHA has been studied because of its potential ability to help control metabolic fuels, mainly glucose and lipids, to help control food intake and energy balance [3-6]. How metabolic sensor compounds signal disruption of energy balance is not understood. It is known that derivatives of p-hydroxycinnamic acid bind with bovine serum albumin [7]. Thus, CHA is potentially important as a tool in investigating the distribution, concentration and metabolism of various drugs in the blood stream. CHA also exhibits antimicrobial activity [8] and is involved in other biologically active roles [7]. Thus, it fits one of criteria employed by us in choosing which Lewis bases to investigate for their possible ability to inhibit cancers, viruses, and bacteria.

CHA is derived from natural sources as from the enzymatic synthesis of various plants as Amaranthus cruentus [9], Chenopodium rubrum [10], and Pterocarya fraxinifolia [11]. Thus, it is part of the green chemistry movement.

Fig 1.

Alpha-cyano-4-hydroxycinnamic acid, CHA (Figure 1), is one of the most widely utilized matrix compounds for MALDI MS [12]. It has been included in a number of different polymers as within dendrimers [13], metal framework coordination polymers [14], and connected as appendices to existing polymers [15], but its inclusion within condensation polymers has not been reported [1].

Here we describe the synthesis of condensation polymers (Figure 2) from the interfacial condensation of CHA with organotin dihalides employing the interfacial polycondensation process and preliminary ability of the polymers to inhibit various cancer cell lines.

Fig. 2

2 Experimental

2.1 Synthesis

Diphenyltin dichloride (1135-99-5), alpha-cyano-4-hydroxycinnamic acid (28166-41-8)), dimethyltin dichloride (753-73-1) 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.

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the CHA (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 ^{o}C). Stirring was begun and a heptane solution (30 ml) containing the organotin dihalide (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 to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

2.2 Physical Characterization

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 2000 to 100,000. Fifty to two hundred shots were typically taken for each spectrum. Addition of the graphite matrix material was accomplished by simply "drawing" on the matrix sample holder assembly and placing the powered polymer sample on top of the graphite drawing.

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000. 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.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human pancreas adenocarcinoma cell line (AsPC-1), human pancreas epithelioid duct carcinoma cell line (PANC-1) or mouse embryo-fibroblast (NIH/3T3) cell line or other cell line. Following a 24 h incubation period, the text 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 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.

3 Results and Discussion

3.1 Synthesis and Molecular Weight

Synthesis was carried out employing classical interfacial polycondensation. Table 1 contains the yield, molecular weight and chain length for the products from reaction between CHA, and the organotin dihalides. The actual form of the CHA employed in the reaction is the monosalt because the acid group is not a sufficiently powerful nucleophile to produce the desired ester. Base, sodium hydroxide, is added forming the salt that is a sufficiently powerful nucleophile producing the ester linkage.

Table 1.

The reaction is rapid (less than 5 seconds stirring time) employing commercially available reactants and the interfacial reaction system that is currently employed in the industrial synthesis of aromatic amides and polycarbonates. Thus, the process can be employed in the production of grams to tons of product. Product yields are good with the smallest percentage yield for the diphenyltin product.

The products are low to high polymers with chain lengths ranging from 70 to 890. There appears to be no trend with respect to chain length or yield. This is not unexpected since the reaction is complex and chain length and yield probably depend on a number of factors including solubility in each phase, rapidity that the reactants enter the reaction zone, and product solubility since the products are collected as precipitates from the reaction system.

3.2 Infrared Spectroscopy

Infrared spectral analysis was carried out for all of the samples over the range of 4000-650 cm^{-1}. All band locations are given cm^{-1}. Infrared spectral analysis is consistent with the proposed structure and with other reported analyses [16-21]. Bands derived from the monomers and polymers from dibutyltin and diphenyltin dichloride reacting with CHA are given in Table 2. The spectra from all the products show bands characteristic of both reactants and new bands for the product assigned to the Sn-O and Sn-O-C(O) linkages (Table 2) [16-21]. A new band for the Sn-O-C tin-ether linkage is found about 1070 and the Sn-O-C(O) linkage for the tin ester linkage is found about 1020. For C-H stretching about 3000, CHCA has bands about 3044 and 2803. Dibutyltin dichloride has bands at 2960, 2927, 2872 and 2858. The polymer shows bands at 3044, 2957, 2923, 2872, 2858 and 2817 showing bands from both the dibutyltin and CHA moieties.

Diphenyltin dichloride shows bands at 3068 and 3051 and the polymer shows bands at 3068, a broad band about 3044 probably containing the 3051 band from the diphenyltin moiety, and bands at 2980 and 2928 derived from CHA consistent with the presence of units derived from both reactants. Bands characteristic of the carboxylic acid about 3300 are either missing or greatly diminished, as expected. A band about 2230 is present in all of the polymer spectra consistent with the presence of the nitrile, CN, unit. Thus, infrared spectroscopy is consistent with the presence of units from both reactants and the formation of new bands consistent with the formation of the expected Sn-O and Sn-O-C(O) linkages.

Table 2.

Because the CHA is not a symmetrical molecule, at least three arrangements can be present (Figure 3) in the polymer backbone. Two of these are symmetrical (a and b) and one is a mixed linkage (c).

Fig 3.

Even here, there are two different arrangements for the carboxylic arrangement (Figure 4). One of these is described as a distorted six-bonded "bridging" structure shown in Figure 4, left where the carbonyl back-bonds on the tin forming two-"bridged" connections to the tin atom. The corresponding "non-bridged" structure is given in Figure 4, right. These arrangements are also referred to as the bridging and linear or non-bridging.

Fig 4.

Infrared spectroscopy is the easiest way to determine the presence of bridged and non-bridging [1,16-21]. Bridging asymmetric carbonyl absorptions are found around 1570. The bridging symmetric carbonyl band is found around 1410-1435. Non-bridging asymmetric carbonyl bands are found about 1600-1690; and the corresponding symmetric carbonyl bands are found about 1350-1370. Results for the products are given in Table 3.

Table 3.

In general, only bands consistent with non-bridging are found. The lone exceptions are for the dimethyltin and diethyltin products that show some bands associated with bridging.

There is also the question of whether it is the symmetrical or non-symmetrical linkage that predominates. In general, bridging is generally associated with the presence of two carboxyl moieties on the same metal atom which would occur with symmetrical linkages about the metal and not the non-symmetrical linkage as shown in Figure 3, right. Since the compounds are generally of the non-bridging geometry as indicated by infrared spectral results, it is probable that the linkages about the tin atom are non-symmetrical (asymmetrical) [1,16-21]. Thus, infrared spectral results are consistent with the proposed structure with the geometry about the metal mainly being of the non-bridging asymmetric type and the units occurring as alternating asymmetric structures.

3.3 MALDI MS

Matrix-assisted desorption/ionization was independently introduced in by Barber and Liu and coworkers in 1981 [22,23]. In 1988 the addition of the laser as the energy source was introduced by Tanaka, Hillenkamp and coworkers [24,25]. This combination resulted in the creation of matrix-assisted laser/ desorption mass spectroscopy, MALDI MS. In theory MALDI MS is suitable for application to nonvolatile samples including a wide range of natural and synthetic polymers. In reality, this has not occurred because of the requirement that matrix molecules must be in intimate contact with the polymer that is being analyzed. This contact is achieved through dissolving together a suitable matrix material with the polymer. Because many polymeric materials are insoluble in volatile solvents this has largely eliminated most synthetic polymers from being suitably analyzed employing MALDI MS.

For over a decade, we and others have been employing MALDI MS for the identification of non-volatile metal and non-metal containing polymers [1,16-21]. This approach is not straight forward MALDI MS but focuses on fragments that are created in the MALDI MS process. It is applicable to soluble and insoluble products so has wide potential for application. This technique has been recently reviewed [26-29].

Graphite has been employed as a matrix agent using various sources for the graphite including simple pencil “lead” [29-33]. This has been recently reviewed [29]. Here we employed simple pencil lead from a number two pencil as our source of graphite. The advantage of graphite is that no major ion fragment clusters are produced below 500 Da and for our experiments this is below the typical mass range studied [29,30]. In the case of other matrixes, it is possible that ion fragment clusters may be formed from the reaction of the matrix with itself and with the organotin moiety derived from the scission of the organotin polymer. This has not been found for the use of graphite [29].

Table 4 and Figure 5 contain the major ion fragment clusters derived from the product of dibutyltin dichloride and CHA.

Fig 5.

Table 4.

Ion fragment clusters to over two units are found.

Tin contains a number of 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. Tables 5 and 6 contain isotopic abundance matches for ion fragment clusters containing one tin atom (Table 5) and two tin atoms (Table 6). The matches are reasonable and consistent with the ion fragment clusters containing one and two tin atoms. In fact, the presence of this isotopic distribution is both an advantage and disadvantage. It is a disadvantage because the intensity of a “single” tin-containing ion is divided into the various isotopic containing fragments diminishing the specific overall intensity caused by that structure. It is an advantage since agreement of the relative intensities of a single tin-containing fragment with its known isotopic natural abundance, given in the tables as the “Standard”, gives greater confidence that the ion fragment cluster does contain tin.

Table 5.

Table 6.

Table 7.

Figure 6 and Table 7 contains the MALDI MS and tentative assignment for the major ions for the product of CHA and dioctyltin dichloride.

Fig 6.

Table 7.

Ion fragment clusters to three repeat units are found (Table 7)

Isotopic matches for selected ion fragment clusters are given in Tables 8 and 9.

Table 8.

Table 9.

The results are consistent with the ion fragment clusters containing one and two tin atoms.

Similar results are found for the other polymers. Chain scission occurs at the heteroatom and carbonyl-carbon sites as shown below and consistent with other studies. CHA remains intact, with the exception of chain scission sites noted above, in agreement with the mildness of MALDI MS [16-21, 26-30].

Fig 7.

3.4 Proton NMR

NMR was conducted employing d-6 DMSO for the various products. Here we describe results for CHA, organotin dichlorides, and the products with CHA. Figure 5 contains the structure indicating proton locations described for the CHA and butyl chain from dibutyltin dichloride. All bands are given in ppm. CHA has seven protons, six omitting the acid proton, in three environments. For CHA these protons are found at H 2 and 6 at 8.95, 8.93; for H 3 and 5 at 6.9 and 6.87; for H 4 at 5.46; and for H 1 at 8.15. The proton at 4 is missing as expected for the polymers. Dibutyltin dichloride shows four bands between 0.85 to 1.6 (α–1.5, β–1.6, γ–1.3, δ–0.85). For the dibutyltin polymer CHA-associated bands associated with H2 and 6 at 8.1 and 7.9; H3 and 5 at 6.91 and 6.89; and H1 at 7.9 and as expected the H4 is absent. For the butyltin chain the bands are found at α 1.52; β 1.57; γ 1.29; δ 0.82. From the diphenyltin dichloride bands are found at 7.31, 7.41, and 7.81 assigned to the phenyl moiety. For the diphenyl polymer bands appear at H2 8.05 and H6 7.90; H3 and H5 at 6.90 and 6.88; H1 at 7.88 and again the H4 is absent as expected. The phenyl-associate bands appear at 7.29, 7.35 and 7.75. For the polymer, bands associated with the metal units are largely unchanged consistent with polymer formation having minimal effect on the NMR. Bands associated with the CHA are varied with movement found for the protons associated with H5 and H6 protons closest to the insertion of the metal-containing moiety whereas the other bands are largely unchanged. These results are consistent with other studies [34].

Fig 8.

3.5 Cell Analysis Results

Cell lines employed in the current study are given in Table 10.

Table 10.

The cells represent a broad range of cancers. The 3T3 cells are formally described as NIH 3T3 cells. Here, we will use both designations to describe this cell line.

Much of our recent effort has been on discovering compounds that inhibit pancreatic cancer because pancreatic cancer does not have a generally accepted "cure". Thus the set includes two widely employed pancreatic cell lines. These are AsPC-1 which is an adenocarcinoma pancreatic cell line and PANC-1 which is an epithelioid carcinoma pancreatic cell line. The pair of breast cancer cell lines deserves special comment. They represent a matched pair of cell lines. The MDA-MB-231 (strain number 7233) cells are estrogen-independent, estrogen receptor 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 [1]. The PC-3 (3465) cells are of interest because this particular prostate cell line is viewed as one of the most resistant of the prostate cancer cell lines.

While different measures have been employed in the evaluation of cell line results the most widely employed involves the concentration, dose, needed to reduce the growth of the particular cell line. Here we will use effective concentration, EC, values. The concentration of a drug, antibody, or toxicant that induces a response halfway between the baseline and maximum after a

specified exposure time is referred to as the 50% response concentration and is given the symbol EC_{50}.

Table 11 contains the EC_{50} values for the current polymers and monomers. Values for cisplatin are included. Cisplatin is among the most widely employed chemodrugs in the treatment of a wide variety of cancers.

Table 11.

EC_{50} values are similar for the polymers compared with cisplatin. Thus, they are significant. There does not appear to be a difference in the ability to inhibit growth for the two breast-associate cell lines. Also, the polymers exhibit decent inhibition of the two pancreatic cancer cell lines as well as the cell lines of the other cancer-associated cells.

In other studies, we observed a marked ability for polymers containing the dibutyltin moiety, followed by those containing the diphenyltin moiety, to exhibit lower EC_{50} values. This is not the case here where there is similarity between the ability to inhibit cell growth as the organotin moiety varies [1].

Another measure of the potential use of compounds 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 cell line. Again, a variety of symbols are employed to describe similar calculations. Here, we will simply employ the term chemotherapeutic index, CI, so that the CI_{50} is then the ratio of the EC_{50} for the NIH/3T3 or WI-38 cells divided by the EC_{50} for the particular test cell. For simplicity, we will simply employ 3T3 to describe the NIH/3T3 cell line.

Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are the NIH/3T3 and WI-38 cell

lines. We have begun comparing these two cell lines as biomarkers to study the effectiveness of compounds to inhibit the growth of various tumor cell lines.

NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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.

We have employed both cell lines in our studies and have only recently begun to compare results to see if there is a difference between the results found for the two cell lines when they are used to evaluate the ability of materials to arrest the growth of various cancer cell lines. Thus, this study has two parts embedded into it. One is the actual ability to control cancer and the second is the comparison of results derived from employing the two most common standard cells.

The CI_{50} values for the monomers and polymers are given in Table 12. For comparison, values for cisplatin are also given.

Table 12.

The CI_{50} values are similar employing either WI-38 or 3T3 cell as the standard. Thus, it is appropriate for this study to employ either in evaluation of the polymers to inhibit cell growth.

CI_{50} values of 2 and greater are considered significant. For the present study, none of the polymers show CI_{50} values of two and greater. Even so, the CI_{50} values for the polymers are significantly better compared with most of the values for cisplatin.

There is not agreement as whether CI_{50} or EC_{50} have greater significance in evaluating the ability to inhibit cell growth. For the present study, the polymers show decent EC_{50} values but no CI_{50} values of two and greater.

4 Conclusions

Organotin poly(ester ethers) are formed in good yield from the interfacial reaction of organotin dihalides with CHA. Infrared spectroscopy shows the formation of new bands derived from the Sn-O and Sn-O(CO) linkages. It also shows that the products exist as alternating Sn-O and Sn-O(CO) linkages. MALDI MS shows formation of ion fragment clusters several units in length. The products show reasonable inhibition of a variety of cancer cell lines including two pancreatic, two breast, one prostate, one lung, and one colon cancer cell lines. The inhibition of the two pancreatic cancer cell lines by the polymers is significantly better compared with cisplatin.

Because the reactions employ commercially available reactants and the interfacial reaction system that is employed commercially to synthesize aramides and polycarbonates, scale up is readily possible from gram to ton amounts.

References

1. C. Carraher, M. R. Roner, J. Organomet. Chem. **751**, 67 (2014)
2. E. Del Prete, T. A. Lutz, E. Scharrer, Physiology Behavior **80**, 489 (2004)
3. M. I. Friedman, E. M. Stricker, Psychol. Rev. **83**, 409 (1976)
4. E. Scharrer, Nutrition **15**, 704 (1999)
5. E. Scharrer, W. Langhans, W., *Control of Body Fat Content* (Smith, Gordon and Co., London, 1990)
6. T. B. Van Itallie, H. R. Kissileff, H. R., Am. J. Clin. Nutr. **38**, 978 (1983)
7. F. Y. Meng, J. M. Zhu, A. R. Zhao, S. R. Yu, C. W. Lin, C-W., J. Luminescence **132**, 1290 (2012)
8. J. Y. Cho, J. H. Moon, K. Y. Seong, K-Y., Biosci. Biotechnol. Biochem. **62**, 2273 (1998)
9. D. Strack, P. Leicht, M. Brokern, V. Wray, L. Grotjahn, Phytochemistry **26**, 2919 (1987)
10. M. Bokern, V. Wray, D. Strack, D., Phytochemistry **26**, 3229 (1987)
11. B. Meurer, V. Wray, R. Wiermann, D. Strack, Phytrochemistry **27**, 839 (1988)
12. F. Settle, *Handbook of Instrumental Techniques for Analytical Chemistry* (Prentice Hall, Upper Saddle River, NJ. 1997)
13. J. Subbi, R. Aguraiuja, R. Tanner, V. Allikmaa, M. Lopp, Eup. Polym. J. **41**, 2552 (2005)
14. H. Li, M. Guo, H. Tian, F. Y. He, G. H. Lee, S. M. Peng, J. Solid State Chem. **179**, 3511 (2006)
15. L. Hua, T. Y. Low, W. Meng, M. B. Chan-Park, S. K. Sze, Analyst **132**, 1223 (2007)
16. C. Carraher, M. Roner, R. Lambert, L. Arroyo, L. Miller, J. Inorg. Orgamomet. Polym. **25**, 1414 (2015)
17. C. Carraher, M. Ayoub, M. Roner, A. Moric, N. Trang, JCAMS, **1**, 65 (2013)

18. C. Carraher, N. Truong, M. Roner, A. Moric Johnson, N. Sookdeo, N. Trang, Inorg. Chimica Acta **423**, 83 (2014)

19. C. Carraher, A. Morrison, M. Roner, A. Moric, N. Trang, J. Inorgomet. Polym. **24**, 182 (2014)

20. A. Zhao, K. Shahi, M. Roner, G. Barot, T. Fiore, C. Pellerito, M. Scopelliti, L. Pellerito, C. Carraher, J. Polym. Mater. **25**, 87 (2008)

21. G. Barot, M. Roner, Y. Naolshima, K. Nagao, K. Shahi, C. Carraher, J. Inorg. Organomet. Polym. **19**, 12 (2009)

22. M. Barber, R. S. Bordoli, R. D. Sedwick, A. N. Tyler, Nature **293**, 270 (1981)

23. L. K. Liu, K. L. Busch, R. G. Cooks, R.G., Analyt. Chem. **53,** 109 (1981)

24. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, T. Matsuo, Rapid Com. Mass Spectrometry **2**, 151 (1988)

24. M. Karas, M., D. Bachmann, F. Hillenkamp, Analytical Chemistry **57**, 2935 (1985)

26. C. Carraher, T. Sabir, C. L. Carraher, *Inorganic and Organometallic Macromolecules* (Springer, NY, 329, 2008)

27. C. Carraher, T. Sabir, C. L. Carraher, J. Polym. Mater. **23**, 143 (2006)

28. C. Carraher, G. Barot, A. Battin, J. Polym. Mater. **26**, 17 (2009)

29. C. Carraher, M. R. Roner, C. L. Carraher, R. Crichton, K. Black, K., J. Macromol. Sci., A. **52**, 867 (2015)

30. C. Carraher, V. Suresh, M. Roner, J. Polym. Mater. **32**, 147 (2015)

31. C. Black, J. Poile, J. Langley, J. Herniman Rapid Comm Mass Spectrom **20,** 1053 (2006)

32. J. Sunner, E. Dratz, Y. C. Chen, Anal Chem **67,** 4335 (1995)

33. E. Berger-Nicoletti, F. Wurm, A. Kilbinger, H. Frey, Macromolecules **40,** 746 (2007)

34. O. Tarzi, H. Nonami, R. Erra-Balsells, J. Mass. Spectrom. **44**, 260 (2009)

Tables

Table 1 Product yield and chain length for the synthesis of organotin poly(ester ethers) from reaction of organotin dihalides with alpha-cyano-4-hydroxycinnamic acid

Organotin Moiety	Percentage Yield	Molecular Weight	Chain Length
Me_2Sn	85	8.0×10^4	180
Et_2Sn	87	4.2×10^5	890
Bu_2Sn	88	3.8×10^4	70
Oc_2Sn	94	1.5×10^5	220
Ph_2Sn	68	2.1×10^5	370

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

Band Assignment	**CHCA**	**Bu_2SnCl_2**	**Bu_2Sn Polymer**	**Ph_2SnCl_2**	**Ph_2Sn Polymer**
OH St	3299				
CH St Aromatic	3044		3044	3068, 3051	3068, 3044
CH Sym St Aliph		2960, 2927	2957, 2923		2980, 2928
CH Asym St Aliph	2803	2872, 2858	2868, 2856, 2817		2800
CN St	2230		2229		2228
C=O St	1669		1670		1670
Ring CC ip St	1589, 1559, 1509, 1435		1595, 1563, 1511, 1440		1587, 1558, 1510, 1440
Sn-Ph St				1480, 1071	1480, 1071
CH_3 Sym St		1463	1463		
C=C St				1432, 1332	1430, 1330
CH_3 Asy Bend		1380	1393		
CH Wag C=C	1370		1370		1372
C-OH St Acid	1320				

Ring CC ip St	1284, 1217, 1167		1288, 1171	1216,	1287, 1217, 1172
Sn-O-C			1081		1071
Sn-O-C(O) St			1017		1021
Ring Breathing				996	997
CH_3 Rock		878	874		
Syn op Bend Ring Hydrogens				729	725
Asy op Bend Ring Hydrogens				691	691

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

Organotin Moiety	Asym Non-bridging	Sym Non-bridging	Asym Bridging	Sym Bridging
Me_2Sn	1602(l)	1349(m)	1567(s)	1434(s)
Et_2Sn	1621(s)	1360(s)	1570(m)	1430(s)
Bu_2Sn	1690(l)	1360(m)		

Oc_2Sn	1647(m)	1360(s)
Ph_2Sn	1669(l)	1362(m)

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

Table 4 Major ion fragments and tentative assignments for the product of dibutyltin dichloride and CHA with graphite as the matrix

Ion Frag. Cluster, Da	**Tent. Assign.**	**Ion Frag. Cluster, Da**	**Tent. Assign.**
551	U+CO	839	$U+Bu_2Sn,CO_2$
589	$U+Na,CO_2$	860	$U+Bu_2Sn,2CO_2,Na$
632	U+CHA-2Bu,O,CO_2	878	$2U-2Bu,CO_2$
647	U+Sn	917	2U-2Bu,O
669	U+Sn,2O	1049	2U
709	U+CHA-2Bu,O	1087	$2U+CO_2$
743	$U+BuSn,CO_2$	1240	2U+BuSn,O
766	$U+ Bu_2Sn$	1277	$2U+ Bu_2Sn$
800	$U+Bu_2Sn,CO_2$		

Table 5 Isotopic abundance match of the ion fragment tentatively assigned to the ion fragment centering about 632 Da and assigned to U+CHA-2Bu,O,CO_2 containing one tin employing graphite as the matrix (Only ion fragment >5% relative abundance are reported.)

Standard		**Found**	
Da	**%-Rel Abund**	**Da**	**%-Rel Abund**
116	45	628	45
117	24	629	26
118	75	630	75
119	26	631	32
120	100	632	100
122	14	634	15
124	17	636	18

Table 6 Isotopic abundance match for an ion fragment cluster containing two tin atoms derived from the product of dibutyltin dichloride and CHA employing graphite as the matrix (Only ion fragments >5% relative abundance are reported.)

Standard		U+Sn,2O	
Da	**% Rel Abu**	**Da**	**% Rel Abu**
232	12	663	14
233	13	664	15
234	43	665	46
235	35	666	35
236	94	667	88
237	51	668	53
238	100	669	100
239	35	670	40
240	81	671	84
242	32	673	36
244	22	675	28

Table 7 Major ion fragments and tentative assignments for the product of dioctyltin dichloride and CHA with added graphite as the added matrix

Ion Frag. Cluster, Da	Tent. Assign.	Ion Frag. Cluster, Da	Tent. Assign.
648	U+O	961	U+ Oc_2Sn,O
742	U+CHA-Oc,CO_2,O	1102	2U-Oc,CO_2
798	U+CHA,Na-CO_2	1255	2U-O
848	U+CHA-CO_2,O	1425	2U+CHA-Oc,O
876	U+CHA-CO_2	1633	2U+ Oc_2Sn,O
893	U+Sn,CHA-CO_2	1994	3U+Sn-O

Table 8 Isotopic abundance match of the ion fragment tentatively assigned to the ion fragment centering about 742 Da and assigned to U+CHA-Oc,CO_2,O containing one tin (Only ion fragments >5% relative abundance are reported.)

Standard		Found	
Da	%-Rel Abund	Da	%-Rel Abund
116	45	738	46
117	24	739	26
118	75	740	76

119	26	741	27
120	100	742	100
122	14	744	16
124	17	746	18

Table 9 Isotopic abundances for two ion fragments derived from reaction of dioctyltin dichloride and CHA containing two tin atoms (for isotopes whose relative abundances are >10%)

Standard for 2 Sn		**U+Sn,CHA-CO_2**		**2U-O**	
232	12	887	12	1249	10
233	13	888	12	1250	11
234	43	889	41	1241	41
235	35	890	38	1252	40
236	94	891	91	1253	90
237	51	892	58	1254	55
238	100	893	100	1255	100
239	35	894	34	1256	31
240	81	895	84	1257	84
242	32	897	36	1259	30
244	22	899	21	1261	24

Table 10 Cell lines employed in the current study

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Table 11 EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values given in ( ) are Standard Deviations for each set of measurements

Sample	**3T3**	***WI-38***	***PANC-1***	***AsPC-1***
Me_2SnCl_2	0.43 (.1)	0.22(.1)	0.80(.1)	0.71(.1)
Me_2Sn/CHA	34(3)	35(4)	33(3)	39(4)
Et_2SnCl_2	0.46(.1)	0.20(.1)	0.48(.1)	0.90(.1)
Et_2Sn/CHA	18(2)	17(2)	16(2)	20(2)
Bu_2SnCl_2	0.20 (.05)	0.20(.05)	>15	>15
Bu_2Sn/CHA	28(3)	26(3)	26(3)	28(3)
Oc_2SnCl_2	0.56(.1)	0.30(.1)	0.85(.1)	0.85(.1)
Oc_2Sn/CHA	29(2)	20(2)	22(2)	21(2)
Ph_2SnCl_2	0.66(.1)	0.25(.1)	0.71(.1)	0.83(.1)
Ph_2Sn/CHA	17(2)	16(2)	17(2)	18(2)
CHA	>60	>60	>60	>60
Cisplatin	15(10)	1,200(19)	1,400 (150)	340(12)

Sample	***PC-3***	***MDA-MB-231***	***HT-29***	***MCF-7***
Me_2SnCl_2	0.51(.1)	0.44(.1)	0.56(.1)	0.66(.1)
Me_2Sn/CHA	39(4)	38(4)	35(3)	33(3)
Et_2SnCl_2	0.61(.1)	0.64(.1)	0.71(.1)	0.77(.1)
Et_2Sn/CHA	20(2)	20(2)	19(2)	19(2)
Bu_2SnCl_2	1.4(1.1)	1.4(1.3)	1.2(.1)	0.7(.06)
Bu_2Sn/CHA	30(3)	27(3)	28(3)	29(3)

Oc_2SnCl_2	0.55(.1)	0.65(.1)	0.65(.1)	0.70(.1)
Oc_2Sn/CHA	22(2)	21(2)	23(2)	23(2)
Ph_2SnCl_2	0.82(.1)	0.76(.1)	0.56(.1)	0.68(.1)
Ph_2Sn/CHA	20(2)	21(2)	23(2)	20(2)
CHA	>60	>60	>60	>60
Cisplatin	1.00	3.00	2.00	1.00(0.1)
	(0.10)	(0.28)	(0.21)	

Table 12 CI_{50} results for values calculated from data given in Table 11

Sample	**EC_{50} *3T3*/ EC_{50}*3T3***	**EC_{50} *3T3*/EC_{50} WI-38**	**EC_{50} 3T3/ EC_{50}*PANC*-1**	**EC_{50} *3T3*/ EC_{50}*ASPc-1***
Me_2SnCl_2	1	2	0.54	0.61
Me_2Sn/CHA	1	0.98	1.1	0.98
Et_2SnCl_2	1	1.2	1	1
Et_2Sn/CHA	1	1.1	1.1	0.90
Bu_2SnCl_2	1	1		
Bu_2Sn/CHA	1	1.1	1.1	1.0

Oc_2SnCl_2	1	1.9	0.66	0.66
Oc_2Sn/CHA	1	1.5	1.3	1.4
Ph_2SnCl_2	1	2.6	0.92	0.80
Ph_2Sn/CHA	1	1.1	1.0	0.98
Cisplatin	1	60	8.8	2.1

Sample	**EC_{50} *3T3*/ EC_{50}*PC-1***	**EC_{50} *3T3*/ EC_{50}*MDA***	**EC_{50} *3T3*/ EC_{50}*HT-29***	**EC_{50} *3T3*/ EC_{50}*MCF-7***
Me_2SnCl_2	0.84	0.98	0.77	0.65
Me_2Sn/CHA	0.89	0.89	0.98	1.1
Et_2SnCl_2	1.1	1.1	0.80	0.86
Et_2Sn/CHA	0.90	0.90	0.98	0.98
Bu_2SnCl_2	0.14	0.14	0.17	0.29
Bu_2Sn/CHA	0.93	1.1	1.0	0.98
Oc_2SnCl_2	1.0	0.86	0.86	0.76
Oc_2Sn/CHA	1.3	1.4	1.3	1.3
Ph_2SnCl_2	0.80	0.87	1.2	0.98
Ph_2Sn/CHA	0.85	0.81	0.74	0.85
Cisplatin	3.0	3.0	1.5	1

Sample	EC_{50} *WI-38*/ EC_{50}*3T3*	EC_{50} *WI-38*/ EC_{50}*WI-38*	EC_{50} *WI-38*/ EC_{50}*PNC-1*	EC_{50} *WI-38*/ EC_{50}*AsPC-1*
Me_2SnCl_2	0.51	1	0.28	0.31
Me_2Sn/CHA	1.1	1	1.1	0.90
Et_2SnCl_2	0.83	1	0.83	0.81
Et_2Sn/CHA	0.98	1	1.1	0.85
Bu_2SnCl_2	1	1		
Bu_2Sn/CHA	0.93	1	1.0	0.98
Oc_2SnCl_2	0.55	1	0.35	0.35
Oc_2Sn/CHA	1	1.5	1.3	1.4
Ph_2SnCl_2	0.38	1	0.35	0.31
Ph_2Sn/CHA	0.98	1	0.98	0.89
Cisplatin	0.02	1	0.15	0.04

Sample	EC_{50} *WI-38*/ EC_{50}*PC-3*	EC_{50} *WI-38*/ EC_{50}*MDA*	EC_{50} *WI-38*/ EC_{50}*HT-29*	EC_{50} *WI-38*/ EC_{50}*MCF-7*
Me_2SnCl_2	0.43	0.50	0.39	0.39
Me_2Sn/CHA	0.90	0.92	1.0	1.1
Et_2SnCl_2	0.91	0.91	0.67	0.71
Et_2Sn/CHA	0.85	0.85	0.89	0.89
Bu_2SnCl_2	0.14	0.14	0.17	0.29
Bu_2Sn/CHA	0.87	0.98	0.93	0.90

Oc_2SnCl_2	0.55	0.46	0.46	0.43
Oc_2Sn/CHA	0.91	0.98	0.87	0.87
Ph_2SnCl_2	0.30	0.33	0.45	0.37
Ph_2Sn/CHA	0.80	0.76	0.70	0.80
Cisplatin	0.05	0.05	0.025	0.02

Figures

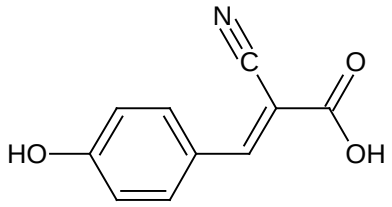


Fig. 1 Alpha-cyano-4-hydroxycinnamic acid, CHA

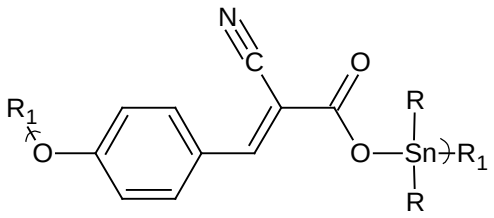
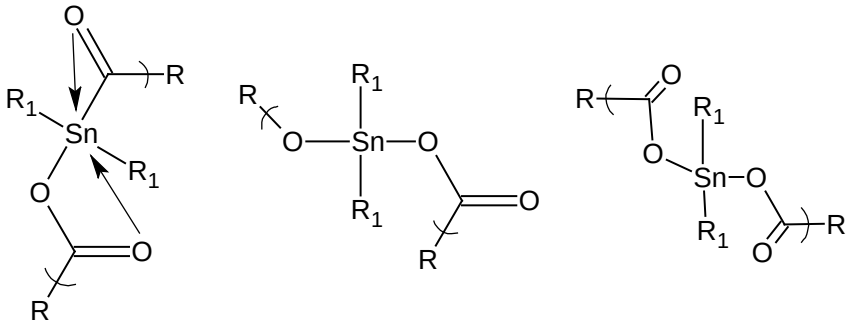


Fig. 2 Repeat unit for the product of alpha-cyano-4-hydroxycinnamic acid, CHA, and organotin dichlorides where R_1 represents simple chain extension

R-O-Sn-O-R R-C(O)-O-Sn-O-C(O)-R R-C(O)-O-Sn-O-R

(a) **(b)** **(c)**

Fig. 3 Arrangements of the CHA moiety about the metal atom



Seven Bonded-Like Linkage Triangular Bipyramidal Mixed Linkage Symmetrical Non-Bridged Linkage

Fig. 4 Geometrical arrangements about the metal atom

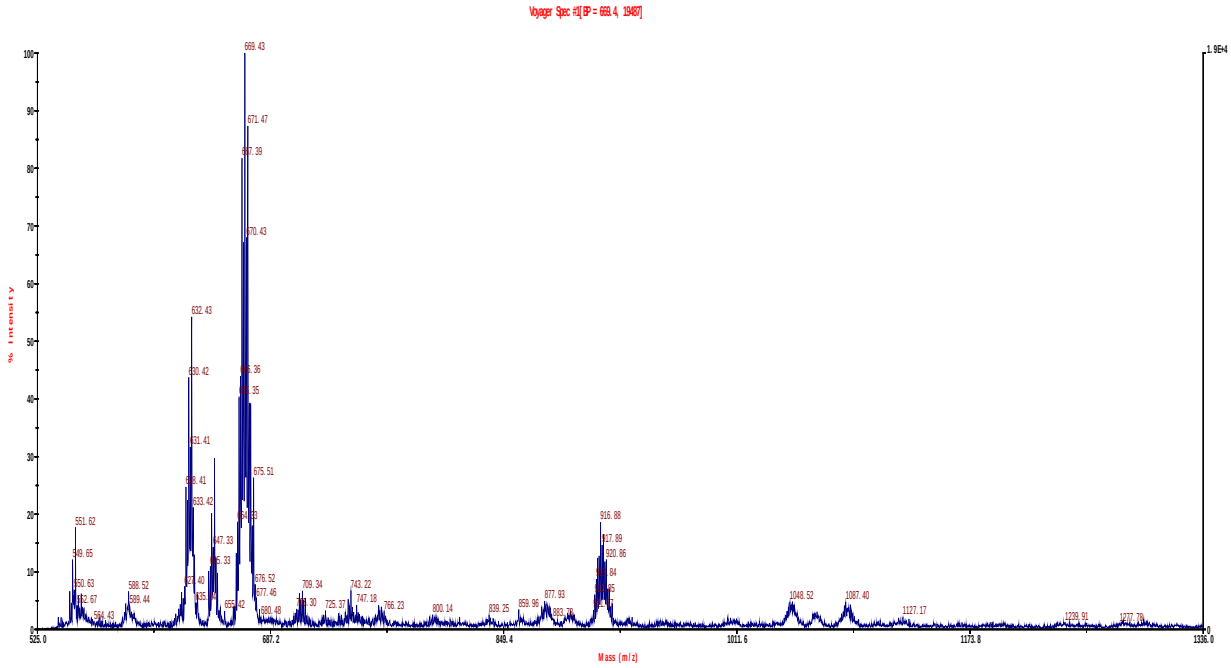


Fig. 5 MALDI MS of the dibutyltin/CHA polymer using graphite as the matrix over the approximate mass range of 500 to 1400 Da

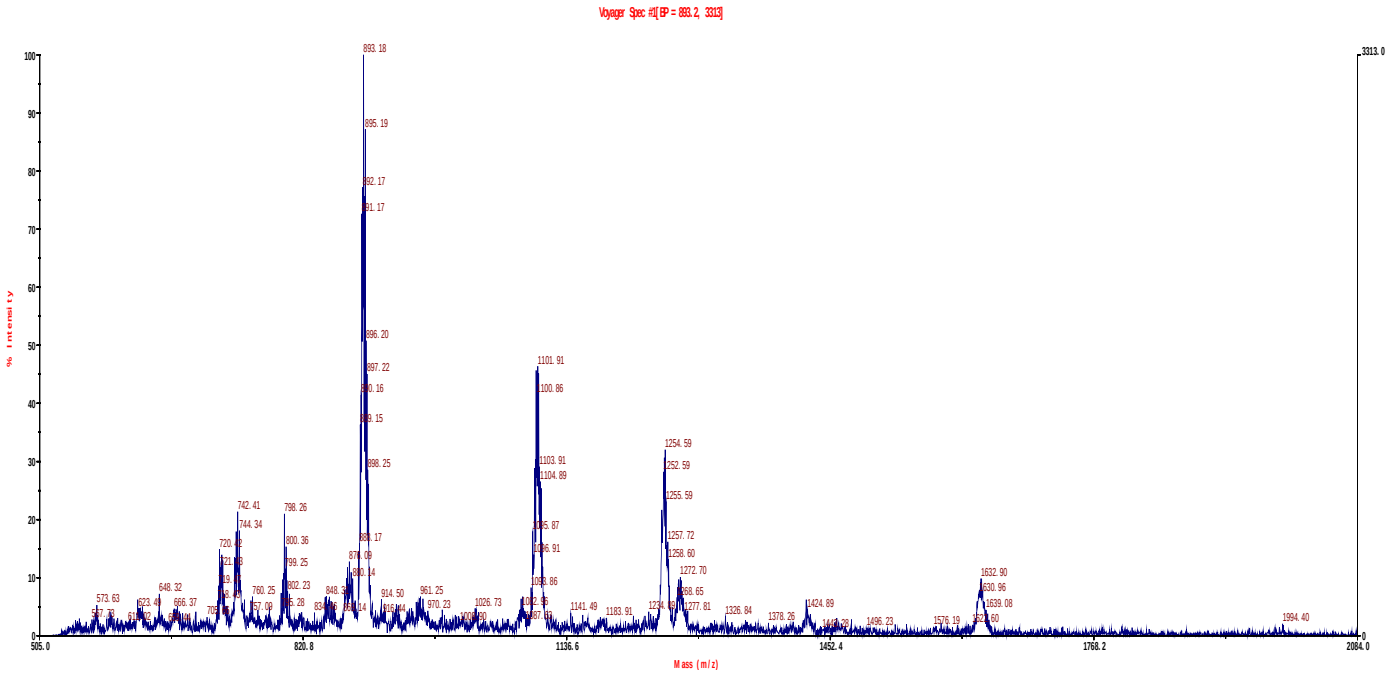


Fig. 6 MALDI MS of the dioctyltin/CHA polymer using graphite as the matrix over the approximate mass range of 500 to 2000 Da

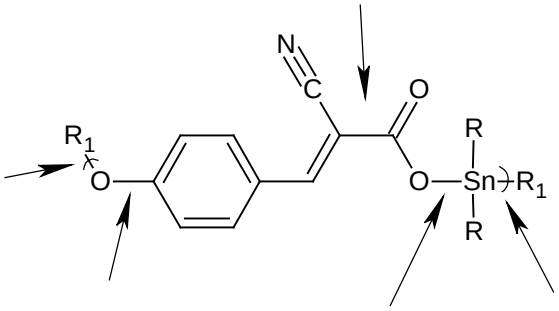


Fig. 7 Repeat unit structure showing preferred sites of bond scission

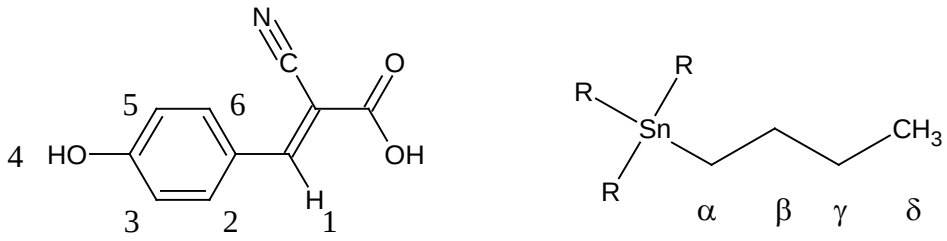


Fig. 8 Structure of CHA and butyl chain of dibutyltin showing protons identified in the proton NMR

CHAPTER 10

SYNTHESIS OF POLY(ETHER ESTERS) FROM REACTION OF ALPHA-CYANO-4-HYDROXYCINNAMIC ACID AND GROUP IVB METALLOCENES[8]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 5 and 6) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Roner, M.R.; Ayoub, M., Crichton, R., Moric-Johnson, A., **Miller, L.** and Black, K.

Charles E. Carraher Jr., Michael R. Roner, Marian Ayoub, Ryan Crichton, Alisa Moric-Johnson, **Lindsey Miller** & Kendra Black (**2016**) Synthesis of poly(ether esters) from reaction of alpha-cyano-4-hydroxycinnamic acid and group IVB metallocenes, Journal of Macromolecular Science, Part A, 53:6, 328-334, DOI: 10.1080/10601325.2016.1165993

[8] Used with permission of Taylor & Francis, 2017

Synthesis of Poly(ether Esters) from Reaction of Alpha-Cyano-4-Hydroxycinnamic Acid and Group IVB Metallocenes

CHARLES E. CARRAHER, JR[1], MICHAEL R. RONER[2], MARIAN AYOUB[1], RYAN CRICHTON[1], ALISA MORIC-JOHNSON[2], LINDSEY MILLER[2], and KENDRA BLACK[1]

[1]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 (carraher@fau.edu)
[2]University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Poly(ether esters) are rapidly synthesized in moderate yield employing the interfacial polycondensation reaction system from the reaction of alpha-cyano-4-hydroxycinnamic acid and Group IVB metallocenes. The products are high polymers. Infrared spectroscopy shows the formation of new bands derived from the M-O and M-O(CO) linkages. It also shows that the products exist as alternating M-O and M-O(CO) linkages. The products show outstanding inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines. EC_{50} values for the polymers are in the nanogram/mL range. The ability to inhibit the cancer cell lines is generally Hf>Zr>Ti. Thus, future synthesis and testing might consider using compounds containing hafnocene and zirconocene in addition to the titanocene moiety.

Keywords: Group IVB-containing polymers, metallocene-containing polymers, hafnocene polymer, zirconocene polymer, titanocene polymers, pancreatic cancer, inhibition of cancer cell lines, interfacial polymerization

*Corresponding author: Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; fax 561-297-2759; words approximately 5,000.

Running title: Synthesis of cyano-4-hydroxycinnamic acid/metallocene polymers

1 Introduction

Part of our recent emphasis involves synthesis of metal-containing polymers to fight cancer and to broaden our knowledge of such materials. A recent focus was on organotin-containing polymers. This was recently reviewed (1). More recently we have begun activity with metallocene-containing

polymers. This has also been reviewed (2). We have continued this effort focusing on the ability of Group IVB metallocenes to become electrically conductive when doped with iodine and because of their biological activity (3-11).

One guiding feature in our effort to suppress various unwanted microbes is coupling the metal-containing moiety which is known to have biological activity with Lewis bases that also offer some biological activity hoping for some synergetic effect from this coupling. It is hoped this coupling results in materials that inhibit the growth or formation of these unwanted microbes at several sites discouraging production of resistant strains.

The anticancer activity of metallocene-containing products is well known since the initial report by Kopf-Maier and Kopf in 1979 of the activity of titanocene dichloride to control cancer cell growth (12). The anticancer activity of such metallocene-containing products has been recently reviewed (13-18).

Alpha-cyano-4-hydroxycinnamic acid, CHA (Figure 1), is known to be biologically active. CHA impairs glucose oxidation by inhibiting pyruvate transport across the mitochondrial membrane (19). Thus, it has been studied because of its potential ability to help control metabolic fuels, mainly glucose and lipids, to help control food intake and energy balance (20-23). The precise mechanism whereby such metabolic sensor compounds signal disruption of energy balance is not well understood. It is also known that p-hydroxycinnamic acid derivatives bind with bovine serum albumin (24). Thus, it is potentially important as a tool in investigating the distribution and concentration and metabolism of various drugs in the blood stream and is also involved in drug stability and toxicity during the chemotherapeutic process. CHA exhibits antimicrobial activity (25). It is also involved in other biologically active roles (24). Thus, it fits one of criteria employed by us in choosing which Lewis bases to investigate for their possible ability to inhibit cancers, viruses, and bacteria. Further, it is derived from natural sources such as from the enzymatic synthesis of various plants such as Amaranthus cruentus (26), Chenopodium rubrum (27), and Pterocarya fraxinifolia (28). Thus, it is part of the green chemistry movement.

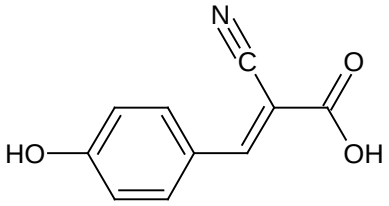


Fig. 1. Alpha-cyano-4-hydroxycinnamic acid, CHA.

Alpha-cyano-4-hydroxycinnamic acid, CHA (Figure 1), is one of the most widely utilized matrix compounds for MALDI MS (29-31). It has been included in a number of different polymers such as within dendrimers (32), metal framework coordination polymers (33), and connected as

appendices to existing polymers (34), but its inclusion within condensation polymers has not been reported except for organotin-containing products (1,35).

Here we describe the synthesis of condensation polymers (Figure 2) from the interfacial condensation of CHA with various Group IVB metallocene dihalides employing the interfacial polycondensation process (Figure 2 for the titanocene moiety). We recently described the use of the analogous organotin products as self-matrixing materials as the initial example of condensation polymers containing a matrix material that allows it to act as its own matrix material (35). In a sister paper (36) we describe the ability of the polymers described in this paper to also self-matrix to obtain MALDI MS. Here we describe the synthesis, structural characterization and initial ability of these polymers to inhibit a wide variety of cancer cell lines.

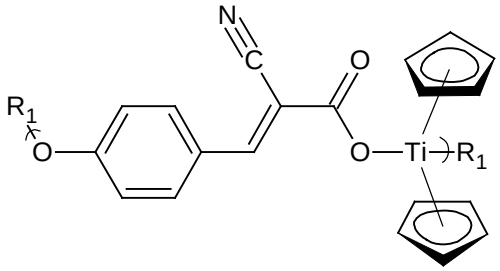


Fig. 2. Repeat unit for the product of alpha-cyano-4-hydroxycinnamic acid, CHA, and titanocene dichloride where R_1 represents simple chain extension.

2 Results and Discussion

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the CHA (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 ^{o}C). Stirring was begun and a chloroform solution (30 ml) containing the metallocene dichloride (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 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), alpha-cyano-4-hydroxycinnamic acid (28166-41-8), and hafnocene dichloride (12116-66-4) acid were purchased from Aldrich Chemical Co., Milwaukee, WS and used as received.

2.2 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 amd Varian 500 MHz spectrometers.

2.3 Cell Testing

The toxicity of each test compound was evaluated with the human pancreas adenocarcinoma cell line (AsPC-1), human pancreas epithelioid duct carcinoma cell line (PANC-1) or mouse embryo-fibroblast (NIH/3T3) cell line or other cell line. Following a 24 h incubation period, the text 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 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.

3. Results and Discussion

3.1 Synthesis and Molecular Weight

Table 1 contains the yield, molecular weight and chain length for the products from reaction between CHA, and the Group IVB metallocene dichlorides. Product yield is moderate and the products are high polymers. The actual form of the CHA employed in the reaction is the monosalt because the acid group is not a sufficiently powerful nucleophile to produce the desired ester. Base, sodium hydroxide, is added forming the salt that is a sufficiently powerful nucleophile producing the ester linkage.

Table 1. Product yield, molecular weight and chain length.

Metallocene Moiety	Percentage Yield	Molecular Weight, Da	Chain Length
Cp_2Ti	32	1.5×10^5	320
Cp_2Zr	37	1.3×10^6	2500
Cp_2Hf	72	1.2×10^6	2000

3.2 Infrared Spectroscopy Analysis

Infrared spectra were taken of the reactants and products over the range of 4000 to 650 cm^{-1}. All bands are given in cm^{-1}. Band assignments are given in Table 2 for CHA, titanocene dichloride and the polymers derived from reaction between the various Group IVB metallocene dichlorides and CHA. Briefly, for all of the products, the C-H aromatic stretch for the C-H in the cyclopentadiene moiety is found near 3100 for the products and from CHA near 3045. Bands from the CHA derived moiety assigned to the C-H asymmetric and symmetric stretching are found in the polymer about 2930, and 2800. Additional bands assigned to wagging, scissoring, and rocking are also found as are a number of combination bands (Table 2).

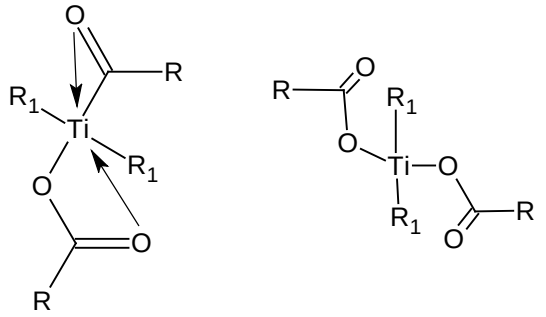
Table 2. Selected infrared bands for the monomers and polymers associated with the CHA Group IVB metallocene polymers.

Band Assignment	CHA	Cp_2TiCl_2	Cp_2Ti/CHA	Cp_2Zr/CHA	Cp_2Hf/CHA
OH St	3299				
CH St Aromatic	3044	3103	3115,3045	3115,3045	3016,3045
CH Sym St Aliph	2930		2930	2930	2915
CH Asym St Aliph	2803		2805	2806	2810
CN St	2230		2232	2220	2224
C=O St	1669		1673	1574	1580
Ring CC ip St	1589,1559, 1509		1590,1565, 1510	1590,1565, 1508	1590,1560, 1506
Cp St		1440	1438	1439	1440
CH Wag C=C; C-C St	1370	1371	1371	1369	1370
C-OH St Acid	1320				
Ring CC ip St	1284,1217, 1167		1287,1216, 1169	1280,1219, 1171	1282,1220, 1170
CH ip Wag		1014	1016	1018	1018

C-H ip Wag	873, 827	875,830	878,817	873,820

Several M-O bands are possible including those from attachment to the R-C(O)-O moieties. Further, there can be a variety of stretching, torsonal, etc. bands associated with the formation of the M-O moiety. The Ti-O asymmetric stretch appears at 1291 in the polymer. The Ti-O symmetric stretch appears at 725. A new band about 1088 is assigned to the Ti-O-C(O) stretch. Thus, bands are present characteristic of the formation of the Ti-O-C(O) linkage. For Zr these bands appear for the polymer at 1290, 724 and 1055 and for the Hf polymer at 1291, 722 and 1055.

There are two different geometries for the carboxylic arrangement (Figure 3). One of these is described as a distorted six-bonded "bridging" structure shown in Figure 3, left where the carbonyl back-bonds on the metal forming two-"bridged" connections to the metal atom. The corresponding "non-bridged" structure is given in Figure 3, right. These arrangements are also referred to as the bridging and linear or non-bridging.



Octahedral Bridged Bonded Tetrahedral Non-Bridged

Fig. 3. Geometrical arrangements about the titanium atom.

Infrared spectroscopy is the easiest way to determine the presence of bridged and non-bridging (1,2). Bridging asymmetric carbonyl absorptions are found around 1550. 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 1350-1370. 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		--	1550(l)	1410(l)
Cp_2Zr	1646(vs)	--	1539(l)	1415(l)
Cp_2Hf	1646(vs)	--	1543(l)	1418(l)

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

Bands associated with nonbonding are small or missing while bands assigned to bridging are large. This is consistent with bridging being the major form of carboxyl-associated bonding about the metal atom. Further, we find that bridging typically occurs when there are two carboxyl groups on the same metal atom so that the products exist within the polymer backbone as mainly symmetrical structures rather than asymmetrical structures (Figure 4). Thus, the polymer chains contain largely alternating repeat units within the polymer chain.

(CO)O-M-O(CO)	O-M-O	O-M-O(CO)
Symmetrical	Symmetrical	Asymmetrical

Fig.4. Possible repeat unit structures about the metallocene atom within the polymer chain.

The largely alternating structure probably results because the CHA contains the alcohol end-group that has some solubility in the organic layer whereas the acid salt has little solubility in the organic layer but relatively high solubility in the aqueous layer allowing for preferential reaction initially at one of the ends resulting in observed repeat unit for the polymer (37-39).

3.3 Proton NMR

NMR was conducted employing d-6 DMSO for the various products. Here we describe results for CHA, metallocene dichlorides, and the products with CHA. Figure 5 contains the structure indicating proton locations described for the CHA. The metallocene dichlorides all have a band about 6.5 (all bands are given in ppm). CHA has seven protons, six omitting the acid proton, in three environments. For CHA these protons are found at H 2 and 6 at 8.95, 8.93; for H 3 and 5 at 6.9 and 6.87; for H 4 at 5.46; and for H 1 at 8.15. The proton at 4 is missing as expected for the polymers. For the titanocene dichloride polymer bands are found at 6.46 and 6.44 for the titanocene moiety; and for the CHA at 8.90 and 8.88; 6.91 and 6.89; and at 8.15. For the zirconocene polymers the bands associate with the metallocene protons are found at 6.55 and 6.54 and from the CHA moiety at 8.95 and 8.95; 6.91 and 6.89; and at 8.16. For the hafnocene polymers bands are found at 6.55 and 6.54; for the CHCA moiety bands are found at 8.96 and 8.94; 6.91 and 6.89; and at 8.15. The NMR bands in the polymer and monomers are similar consistent with polymer formation having minimal effect on the NMR. These results are consistent with other studies (2,40).

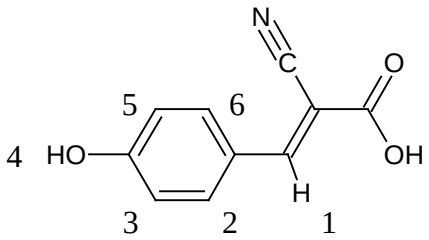


Fig. 5. Structure of CHA showing protons identified in the proton nmr.

3.4 Cell Analysis Results

The cell lines employed in the current study are given in Table 4.

Table 4. Cell lines employed in the current study.

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

The cells represent a broad range of cancers. The 3T3 cells are formally described as NIH 3T3 cells. Here, we will use both designations to describe this cell line.

Much of our recent effort has been on discovering compounds that inhibit pancreatic cancer because pancreatic cancer does not have a generally accepted "cure". Thus the set includes two widely employed pancreatic cell lines. These are AsPC-1 which is an adenocarcinoma pancreatic cell line and PANC-1 which is an epithelioid carcinoma pancreatic cell line. The two tested pancreatic cancer cells are human cell lines widely employed in testing for inhibition of pancreatic cancer. 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 metal-containing polymers we found there was a marked difference between the ability to inhibit the two cell lines dependent on polymer structure (1, 41,42). The PC-3 (3465) cells are of interest because this particular prostate cell line is viewed as the most resistant of the readily available prostate cancer cell lines.

While different measures have been employed in the evaluation of cell line results the most widely employed involves the concentration, dose, needed to reduce the growth of the particular

cell line. Here we will use effective concentration, EC, values. The concentration of a drug, antibody, or toxicant that induces a response halfway between the baseline and maximum after a specified exposure time is referred to as the 50% response concentration and is given the symbol EC_{50}.

Table 5 contains the EC_{50} values for the current polymers and monomers. Values for cisplatin are included. Cisplatin is among the most widely employed chemodrugs in the treatment of a wide variety of cancers.

Table 5. EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values given in ( ) are standard deviations for each set of measurements.

Sample	3T3	*WI-38*	*PANC-1*	*AsPC-1*
Cp_2TiCl_2	1.8(.2)	2.2(.1)	0.45(.3)	0.51(.05)
Cp_2Ti/CHA	2.9(.3)	2.3(.3)	0.071(.04)	0.055(.007)
Cp_2ZrCl_2	1.8(.2)	0.94(.1)	0.38(.3)	0.44(.05)
Cp_2Zr/CHA	2.2(.3)	1.6(.001)	0.0016(.001)	0.00097 (.0001)
Cp_2HfCl_2	1.7(.2)	1.2(.2)	0.22(.2)	0.18(.01)
Cp_2Hf/CHA	2.8(.3)	1.9(.2)	0.0011(.0005)	0.0058(.001)
CHA	>60000	>60000	>60000	>60000
Cisplatin	15(10)	1,200 (19)	1,400 (150)	340(12)

Sample	*PC-3*	*MDA-MB-231*	*HT-29*	*MCF-7*
Cp_2TiCl_2	0.48(.05)	0.35(.05)	0.54(.06)	0.47(.05)
Cp_2Ti/CHA	0.059 (.005)	0.067(.008)	0.034(.004)	0.039 (.005)
Cp_2ZrCl_2	0.25(.03)	0.27(.03)	0.31(.02)	0.33(.05)
Cp_2Zr/CHA	0.0011 (.0002)	0.0013 (.0002)	0.00094 (.0001)	0.0018 (.003)
Cp_2HfCl_2	0.25(.03)	0.27(.03)	0.31(.02)	0.33(.05)
Cp_2Hf/CHA	0.00097 (.0001)	0.00065 (.00008	0.0011 (.0001)	0.00085 (.0001)
CHA	>60000	>60000	>60000	>60000
Cisplatin	1.00 (0.10)	3.00 (0.28)	2.00 (0.21)	1.00(0.1)

EC_{50} values for the metallocene dichlorides are generally a decade lower than for cisplatin for all of the cancer cell lines and for the healthy cell line standard W38 the difference is much greater. In comparison with the polymers, the EC_{50} values for the polymers are generally much less ranging from about a hundred fold less to greater than a thousand fold less compared to cisplatin. Thus, the polymers and metallocene monomers in every case show a much greater toxicity towards the cancer cell lines in comparison to cisplatin. Further, the polymers exhibit a much enhanced inhibition towards the cancer cells compared to the metallocene dichlorides. Since the Lewis base itself shows no inhibition towards any of the cells tested (to the concentration limits employed) it is the combination that is responsible for the activity.

There does not appear to be a difference in the ability to inhibit growth for the two breast-associate cell lines. Also, the polymers exhibit good inhibition of the two pancreatic cancer cell lines as well as the cell lines of the other cancer-associated cells.

Another measure of the potential use of compounds 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, we will simply employ the term chemotherapeutic index, CI, so that the CI_{50} is then the ratio of the EC_{50} for the NIH/3T3 or WI-38 cells divided by the EC_{50} for the particular test cell. For simplicity, we will simply employ 3T3 to describe the NIH/3T3 cell line.

Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are the NIH/3T3 and WI-38 cell lines. NIH/3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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.

We have employed both cell lines in our studies and have only recently begun to compare results to see if there is a difference between the results found for the two cell lines when they are used to evaluate the ability of materials to arrest the growth of various cancer cell lines. Thus, this study has two parts embedded into it. One is the actual ability to control cancer and the second is the comparison of results derived from employing the two most common standard cells.

The CI_{50} values for the monomers and polymers are given in Table 6. For comparison, values for cisplatin are also given.

Table 6. CI_{50} results for values calculated from data given in Table 2.

Sample	EC_{50} *3T3*/ EC_{50}*3T3*	EC_{50} *3T3*/EC_{50} WI-38	EC_{50} 3T3/ EC_{50}*PANC*-1	EC_{50} *3T3*/ EC_{50}*ASPc-1*
Cp_2TiCl_2	1	0.82	4.0	3.5
Cp_2Ti/CHA	1	1.3	41	53
Cp_2ZrCl_2	1	1.9	4.7	4.1
Cp_2Zr/CHA	1	1.4	140	2300
Cp_2HfCl_2	1	1.4	7.7	6.7
Cp_2Hf/CHA	1	1.5	2500	480
Cisplatin	1	0.13	0.11	0.44

Sample	EC_{50} *3T3*/ EC_{50}*PC-1*	EC_{50} *3T3*/ EC_{50}*MDA*	EC_{50} *3T3*/ EC_{50}*HT-29*	EC_{50} *3T3*/ EC_{50}*MCF-7*
Cp_2TiCl_2	4.0	5.1	3.3	3.8
Cp_2Ti/CHA	49	43	85	74
Cp_2ZrCl_2	7.2	6.7	5.8	5.4
Cp_2Zr/CHA	2000	1700	2300	120
Cp_2HfCl_2	6.8	6.3	5.5	5.2
Cp_2Hf/CHA	2900	4300	2500	3300
Cisplatin	15	5	7.5	15

Sample	EC_{50} *WI-38*/ EC_{50}*3T3*	EC_{50} *WI-38*/ EC_{50}*WI-38*	EC_{50} *WI-38*/ EC_{50}*PNC-1*	EC_{50} *WI-38*/ EC_{50}*AsPC-1*
Cp_2TiCl_2	1.2	1	4.9	4.3
Cp_2Ti/CHA	0.79	1	32	42
Cp_2ZrCl_2	0.52	1	2.5	2.1
Cp_2Zr/CHA	1.4	1	1000	1600

Cp_2HfCl_2	5.5	1	5.5	6.7
Cp_2Hf/CHA	0.68	1	1700	330
Cisplatin	80	1	0.86	3.5

Sample	EC_{50} *WI-38*/ EC_{50}*PC-3*	EC_{50} *WI-38*/ EC_{50}*MDA*	EC_{50} *WI-38/* EC_{50}*HT-29*	EC_{50} *WI-38*/ EC_{50}*MCF-7*
Cp_2TiCl_2	4.6	6.3	4.1	4.7
Cp_2Ti/CHA	39	34	68	59
Cp_2ZrCl_2	3.8	3.5	3.0	2.8
Cp_2Zr/CHA	1500	1200	1700	1200
Cp_2HfCl_2	4.8	4.4	3.9	3.6
Cp_2Hf/CHA	2000	2900	1700	2200
Cisplatin	1200	400	600	1200

The CI_{50} values are similar employing either WI-38 or 3T3 cell as the standard. Thus, it is appropriate for this study to employ either in evaluation of the polymers to inhibit cell growth.

CI_{50} values of 2 and greater are considered significant. With the exception of the pancreatic cancer cell lines, the CI values for cisplatin are higher than for the metallocene dichlorides. The CI values for the polymers are also larger compared with the metallocene dichlorides. In fact, the CI_{50} values for the polymers are also larger than CI_{50} values for cisplatin. In comparison to the metallocene dichlorides the CI_{50} values for the polymers are generally a decade greater and in some cases over one thousand times larger.

Another general trend involves the EC_{50} and CI_{50} values and the nature of the metals for the polymers. In general, the EC_{50} values follow the order of Ti>Zr>Hf and consequently the CI_{50} values follow the order Hf>Zr>Ti so that in terms of treating cancer cell lines, the Hf and Zr polymer favor treatment with the Ti polymer. The Zr and Hf values are similar while the values for the Ti are considerably different. Thus, while the titanocene dichloride was chosen for clinical studies (as will be noted following), from our system it would be the Zr and Hf polymers that would be chosen based on the present study. Chemically, it is known that this family of metals is

the closest in chemical behavior of all of the families of elements to such an extent that it is difficult to separate the three metals.

Another concern involves the toxicity of the metal-containing moieties. The toxicity of the metal-containing units varies with the precise compounds as well as mode of measurement and test animal. For cisplatin the LD_{50} are as follows-rat oral 25.8 mg/kg; rat intravenous 8.0 mg/kg; mouse oral 32.7 mg/kg; and mouse intravenous 11 mg/kg (Pfizer Material Safety Data Sheet). For titanocene dichloride the LD_{50} values are rat intraperitoneal 25 mg/kg and mouse intravenous 180 mg/kg (TCI America Material Safety Data Sheet). For zironocene dichloride the rat LD_{50} intraperitoneal is 30 mg/kg (TCI America Material Safety Data Sheet). For hafnocene dichloride the LD_{50} is given as intravenous mouse at 100 mg/kg (TCI America MSD). While exact matches are not available, it appears that cisplatin has lower LD_{50} values, is more toxic, than any of the metallocene dichlorides by about three fold.

Other than platinum-containing small molecules, only titanocene dichloride has undergone clinical studies with humans. Following briefly describes some of these results.

In preclinical studies titanocene dichloride inhibited ovarian cancer cell lines A2780 CP3 that were twenty fold resistant to cisplatin while the cell line was only about two and a half times resistant to titanocene dichloride indicating an absence of cross-resistance between the two metal containing drugs (43). This is consistent with the finding for in vivo studies where titanocene dichloride showed much greater ability to inhibit cisplatin resistant human ovarian cancer xenografts compared to cisplatin. Further, titanocene dichloride largely overcame cisplatin resistance for the A2780CP and CH1cisR ovarian cancer cell lines in bcl-2 and p53 transfectants of A2780 cells (44).

A number of studies are consistent with DNA-metallocene interactions, including titanocene dichloride, zirconocene dichloride, and hafnocene dichloride, being a major determinant in the anticancer activity of these materials (45).

There is a difference between structural dependencies of our compounds and many metallocenes. It was concluded by some studies that the metallocene dichlorides themselves required liability of the chlorides for activity but in the study of similar polymers made by us our compounds exhibit their activity as polymers rather than control delivery of small units (1,46) . Other studies have demonstrated anticancer activity when the halides are replaced so these conclusions are premature (47).

Titanocene dichloride underwent Phase I clinical trials. The trials indicated a dose-limiting side effect associated with nephrotoxicity and a number of unwanted physical side effects including nausea, reversible metallic taste, pain during infusion, hypoglycemia, with these features undesirable. Counter, the absence of an effect on proliferative activity of the bone marrow, generally a dose-limiting side effect, was positive. Some phase II clinical trials were undertaken with patients with breast metastatic carcinoma (48) and advanced renal cell carcinoma (49). Unfortunately, low activity discouraged further clinical study.

Of interest is that our polymers can be readily made in the gram to ton amount since the synthesis employs commercially available materials using the interfacial polymerization system that has been employed commercially to make polycarbonates and aramids (28-30).

The polymers in the present study show much lower concentrations for activity and higher CI_{50} values compared with cisplatin and the Group IVB metallocene dichlorides. Thus, they represent a major potential new group of anticancer drugs. Further, they exhibit great inhibition of the two tested pancreatic cancer cell lines. Since the activity against the two pancreatic cancer cell lines is similar, they may offer a broad range of activity against other pancreatic cancer cell lines.

4. Conclusions

Polyether esters are formed within 15 seconds in moderate yield employing the aqueous (classical) interfacial polycondensation system. The products are high polymers. Synthesis occur employing commercially available reactants and reaction system allowing for ready scale-up from gram to ton amounts. Infrared spectroscopy shows the formation of new bands assigned to M-O and M-O(CO) linkages. IR also shows that the geometrical structure about the metal atom is bridged with respect to the carboxyl unit. This is consistent with the polymer chain having alternating O-M-O and (CO)O-M-O-(CO) units. NMR is consistent with the presence of moieties from both reactants.

Other than platinum-containing drugs related to cisplatin, the only other metal-containing drug to undergo extensive human anticancer testing are monomeric titanocene-associated materials. A major problem associated with titanocene drugs is their lack of decent solubility. The current polymers exhibit good solubility in dimethyl sulfoxide, DMSO. The polymers show superior ability to inhibit call growth compared to the reactants and to cisplatin, a widely employed anticancer drug. The metal-center order of inhibition is Hf>Zr>Ti with the hafnocene and zirconocene polymers showing about a one hundred fold better inhibition of the cancer cell lines compared with the analogous titanocene polymer. Thus, future synthesis and testing might consider using compounds containing hafnocene and zirconocene in addition to titanocene-containing compounds.

References

1. Carraher, C., Roner, M. R. (2014) J. Organomet. Chem., 751, 67–82.
2. Carraher, C., J. (2005) Inorg. Organomet. Polym., 15, 121-145.
3. Carraher, C., Battin, A., Roner, M. R. (2011) J. Functional Biomaterials, 2, 18-30.
4. Battin, A., Carraher, C., J. (2008) Polym. Mater., 25, 23-33.
5. Carraher, C., Battin, A., Roner, M. R. (2012) J. Inorg. Organomet. Polym., 23, 61-73.
6. Roner, M. R., Carraher, C., Shahi, K., Ashida, Y., Barot, G. (2009) BMC Cancer, 9, 358-366.
7. Carraher, C., Roner, M. R., Shahi, K., Ashida, Y., Barot, G. (2007) J. Polym. Mater., 24, 357-

369.
8. Carraher, C., Carraher, S. M., Stewart, H. (2011) J. Marcromol. Sci., 28, 287-301.
9. Sabir, T., Carraher, C. (2005) J. Polym. Mater., 22, 449-458.
10. Carraher, C., Chamely-Wllk, D., Carraher, S. M., Barot, G., Stewart, H. H., Learned, W. (2007) J. Polym. Sci., 24, 149-162.
11. Carraher, C., Roner, M R., Reckleben, L., Black, K., Frank, J., Crichton, R., Russell, F., Moric-Johnson, A., Miller, L. (in press) J. Macromol. Sc.
12. Kopf, H., Kopf-Maier, P. (1979) Angew. Chem., Int. Ed. Engl. 18, 477-478.
13. Melendez, E. (2012) Inorganica Chim. Acta, 393, 36-52.
14. Gasser, G., Ott, I., Metzler-Nolte, N. (2011) J. Med. Chem., 54, 3-25.
15. Gomez-Ruiz, S., Maksimovic-Ivanic, D., Mijatovic, S., Kaluderovic, G. (2012) Bioinorganic Chem., Applications, ID#140284.
16. Abeysinghe, P. M., Harding, M. M. (2007) Dalton Trans., 3474-3482.
17. Kostova, I. (2009) Anti-Cancer Agents Med. Chem., 9, 827-842.
18. Olszewski, U., Hamilton, G. (2010) Anti-Cancer Agents in Med. Chem., 10, 302-311.
19. Del Prete, E., Lutz, T. A., Scharrer, E. (2004) Physiology Behavior, 80, 489-498.
20. Friedman, M. I., Stricker, E. M. (1976) Psychol. Rev., 83, 409-431.
21. Scharrer, E. (1999) Nutrition, 15, 704-714.
22. Scharrer, E., Langhans, W. (1990) Control of Body Fat Content, Smith, Gordon and Co., London.
23. Van Itallie, T. B., Kissileff, H. R. (1983) Am. J. Clin. Nutr., 38, 978-988.
24. Meng, F-Y., Zhu, J-M., Zhao, A-R., Yu, S-R., Lin, C-W. (2012) J. Luminescence, 132, 1290-1298.
25. Cho, J-Y., Moon, J-H., Seong, K-Y. (1998) Biosci. Biotechnol. Biochem., 62, 2273-2276.
26. Strack, D., Leicht, P., Brokern, M., Wray, V., Grotjahn, L. (1987) Phytochemistry, 26, 2919-2922.
27. Bokern, M., Wray, V., Strack, D. (1987) Phytochemistry, 26, 3229-3231.
28. Meurer, B., Wray, V., Wiermann, R., Strack, D. (1988) Phytrochemistry, 27, 839-843.
29. Carraher, C. (2014) Polymer Chemistry, Taylor & Francis, NY.
30. Carraher, C. (2013) Introduction to Polymer Chemistry, CRC, Boca Raton, FL.
31. Settle, F. (1997) Handbook of Instrumental Techniques for Analytical Chemistry, Prentice Hall, Upper Saddle River, NJ.
32. Subbi, J., Aguraiuja, R., Tanner, R., Allikmaa, V., Lopp, M. (2005) Eup. Polym. J., 41, 2552-2560.
33. Li, H., Guo, M., Tian, H., He, F-Y., Lee, G-H., Peng, S-M. (2006) J. Solid State Chem., 179, 3511-3514.
34. Hua, L., Low, T. Y., Meng. W., Chan-Park, M. B., Sze, S. K. (2007) Analyst, 132, 1223-1230.
35. Carraher, C., Suresh, V., Roner, M. R. (2015) J. Chin. Adv. Mater. Soc., 3, 32-44.
36. Carraher, C., Roner, M. R., Ayoub, M., Crichton, R., Black, K. (in press) J. Macromol. Sci.
37. Carraher, C., Lee, J. L. (1975) J. Macromol. Sci.-Chem., A9, 191-198.
38. Carraher, C., Bajah, S. T. (1973) Polymer (Br.) 14, 42-44.
39. Carraher, C., Bajah, S. T. (1974) Polymer (Br.) 15, 9-12.
40. Tarzi, O., Nonami, H., Erra-Balsells, R. (2009) J. Mass. Spectrom. 44, 260-277.

41. Carraher, C., Roner, M., Shahi, K., Barot, G. (2011) Materials, 4, 801–815.
42. Carraher, C., Roner, M. R., Ayhoub, M., Pham, N., Moric-Johnson, A. (2015) Polym. Mater. Polym. Biomat., 64, 311-319.
43. Harstrick, A., Schmoll, H., Sass, G., Poliwoda, H., Rustum, Y. (1993) Eur. J. Cancer, 29A(7), 1000-1002.
44. Christodoulou, C. V., Eliopoulos, A. G., Young, L. S., Hodgkins, L, Ferry, D. R., Kerr, D. J., Br. J. (1998) Cancer, 77(12), 2088-2097.
45. Murray, J. H., Harding, M. M. (1994) J. Med. Chem., 37, 1936-1941.
46. Harding, M. M., Mokdsi, G. (2000) Curr. Med. Chem., 7, 1289-1303.
47. Abeysinghe, P. M., Harding, M. M. (2007) Dalton Trans., 3474-3482.
48. Kroger, N., Kleeberg, U. R., Mross, K., Edler, L., Sab, G., Hossfeld, K. (2000) Onkologie, 23, 60-62.
49. Lummen, G., Sperling, H., Luboldt, H., Otto, T., Rubbe, H. (1998) Cancer Chemotherapy pharmacology, 42(5), 415-421.

CHAPTER 11

SYNTHESIS, STRUCTURAL CHARACTERIZATION AND PRELIMINARY CANCER CELL LINE RESULTS FOR POLYMERS DERIVED FROM REACTION OF TITANOCENE DICHLORIDE AND VARIOUS POLY(ETHYLENE) GLYCOLS[9]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 8 and 9) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Roner, M.R.; Reckleben, L.; Black, K.; Frank, J.; Crichton, R.; Russell, F.; Moric-Johnson, A. and **Miller, L**

Charles E. Carraher Jr., Michael R. Roner, Lisa Reckleben, Kendra Black, Jessica Frank, Ryan Crichton, Floyd Russell, Alisa Moric-Johnson & **Lindsey Miller** (**2016**) Synthesis, structural characterization and preliminary cancer cell line results for polymers derived from reaction of titanocene dichloride and various poly(ethylene glycols), Journal of Macromolecular Science, Part A, 53:7, 394-402, DOI: 10.1080/10601325.2016.1176440

[9] Used with permission of Taylor & Francis, 2017

Synthesis, Structural Characterization and Preliminary Cancer Cell Line Results for Polymers Derived from Reaction of Titanocene Dichloride and Various Poly(ethylene glycols)

Charles E. Carraher, Jr.,[a] Michael R. Roner,[b] Lisa Reckleben,[a] Kendra Black,[a] Jessica Frank,[a] Ryan Crichton,[a] Floyd Russell,[a] Alisa Moric-Johnson,[b] Lindsey Miller[b]

[a]Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 and Florida Center for Environmental Studies, Palm Beach Gardens, FL 33410, carraher@fau.edu***;*** *[b]University of Texas Arlington, Department of Biology, Arlington, TX 76010. roner@uta.edu*

Abstract

We describe the initial synthesis of DMSO and water-soluble titanocene polyethers through reaction of the titanocene dichloride and various poly(ethylene glycols) employing commercially available reactants. Infrared, proton NMR, and MALDI MS results are consistent with the titanocene-containing polyether structure. The materials are polymeric with chain lengths ranging from 1500 to 4. They exhibit good inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines.

Keywords: metal containing polymers; PEG polymers; titanocene-containing polymers; cancer; pancreatic cancer; water soluble polymers, MALDI MS

Contact Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; Fax: 561-297-2759

1. Introduction

We have synthesized a wide variety of metal-containing polymers. Our efforts have been recently reviewed for organotin-containing (1, 2), platinum-containing (3), Group VA metals (4), and Group IVB metallocene containing polymers (5). Here our focus will be on the synthesis of titanocene-containing polyethers. We initially published the synthesis of titanocene-containing polymers in 1971 (6) and shortly thereafter the formation of titanocene polyethers (7) analogous to those describe in the present paper but not based on reaction with poly(ethylene glycol) diols. Recently, this effort emphasized the synthesis of titanocene-containing polymers as electrically-conductive materials (8-10).

Titanocene dichloride and related products are distorted tetrahedrals in geometry. While they have been employed in the formation of many materials, their largest use is as soluble stereoregular catalysts allowing the synthesis of a wide variety of stereoregular polymers including polyethylenes and polypropylenes (11, 12). Cotton and Wilkinson (13) describe Group IVB metallocene dichlorides as 9-coordinate bonding species (the hybrid orbitals being derived from one-s, three-p, and five-d orbitals). Each pi-Cp ring involves three hybrid orbitals. The three remaining orbitals consist of two equivalent spd(x^2-y^2), dz^2 orbitals (overlapping with the two chloride atoms) and one sp orbital which is vacant and believed to be responsible for the catalytic activity of the Group IVB metallocenes (11, 12).

The ability of titanocene-containing small molecules to inhibit cancer cell growth is well known and the mechanistic picture has been recently reviewed and found to be different than for cisplatin (14-19). In fact, titanocene dichloride was the first-non-platinum metal-containing compound to undergo clinical trial (20). 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 (20, 21) and is different than that for cisplatin. Having the mechanism different than that for cisplatin is an advantage since it allows the metallocenes to be part of a "mixture of drugs" delivered to patients that will intersect cancer growth at different sites.

Because of the known anticancer activity for titanocene-containing small molecules we decided to focus on the potential anticancer activity of polymers containing the titanocene moiety. We have started synthesizing various titanocene-containing polymers and found that they indeed exhibit good anticancer activity against a wide variety of cancer cell lines (22, 23).

The initial formation of water soluble organotin polymers was recently effected through the reaction of the organotin dihalides with various poly(ethylene glycols) (Figure 1) (24-26). These polymers exhibited good inhibition of a variety of cancer cell lines (2).

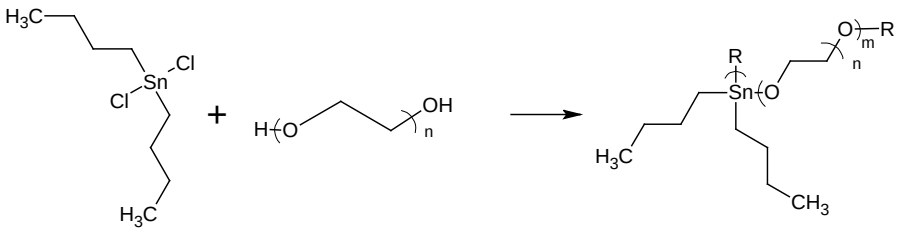


Figure 1. Repeat unit for the water-soluble organotin polyethers where R represents simple chain extension; "n" represents the number of ethylene oxide units; and "m" is the average number of repeat units.

A major problem with the titanocene containing monomeric materials is their lack of solubility. This hinders continued efforts to synthesize and study potential anticancer drugs based on the presence of the titanocene moiety. Here we describe the synthesis of DMSO and water-soluble titanocene-containing polyethers synthesized through reaction with a variety of hydroxyl-terminated poly(ethylene glycols) (Figure 2). Poly(ethylene glycol) (also called poly(ethylene oxide), PEG, is considered to be non-toxic and currently employed in a number of medical-related treatments including as pill coatings and in many laxatives (27-30). It is intentionally attached to many materials including drugs to assist in their water-solubility. When attached to certain protein-medications they allow the drug a longer-activity with reduced toxicity (31). Here, we emphasize its incorporation in the polymer backbone through reaction of the end-groups with the Lewis acid dihalide as shown below for the formation of titanocene polyethers with hydroxyl-capped PEGs where "n" is simply the molecular weight of the PEG divided by 44, the molecular weight of the repeat unit, CH_2CH_2O, for PEG.

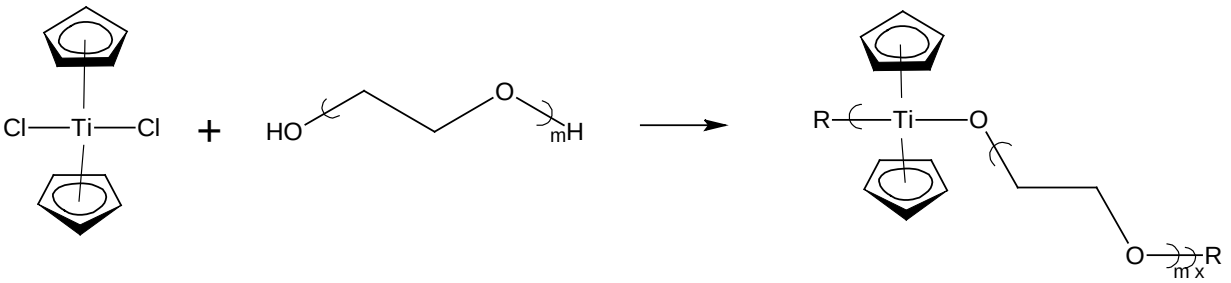


Figure 2. Repeat unit for the proposed unit structure for the reaction between titanocene dichloride and poly(ethylene glycols) where R represents simple chain extension; "n" represents the number of ethylene oxide units; and "m" is the average number of repeat units.

2. Experimental

2.1. Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (50 ml) containing the PEG (0.00100 mol) and sodium hydroxide (0.0020 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^{o}C). Stirring was begun and a chloroform solution (50 ml) containing the titanocene dihalide (0.00100 mol) was rapidly added (about 3-4 sec) through a hole in the jar lid using a powder funnel. The resulting solution was blended for 15 sec. The brownish 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) and the various poly(ethylene glycols) (25322-68-3) were purchased from Aldrich Chemical Co., Milwaukee, WS. The reactants were used as received.

2.2. Physical Characterization

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.

Infrared spectra were recorded using a Mattson galaxy Model 4020 FTIR. Softening point determinations were determined using a Fisher Mel-Temp apparatus equipped with a thermometer calibrated to 300°C. A DuPont 951 Thermogravimetric Analyzer was employed to obtain thermograms of the products in air and nitrogen (inert atmosphere). The gas flow rate was about 70 mL/min. The heating rate was 20°C/min. Elemental analysis for titanium was carried out using about 0.04 g of sample placed in a ceramic crucible that has been dried to constant weight. Approximately 5 drops of perchloric acid was added to the product and the crucible and contents were heated slowly for the initial twenty minutes and then heated vigorously for a total of three hours. After this, if the residue was not totally white, heating was continued with the addition of 3 more drops of perchloric acid and heating continued for two additional hours or until the residue was white as titanium IV oxide is.

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the polymers dissolved in DMSO or water. 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 in d_6 DMSO employing Varian Inova 400 MHz and Varian 500 MHz spectrometers.

2.3. Cell Testing

The toxicity of each test compound was evaluated using a variety of cancer cell lines and with human normal embryonic lung fibroblast (WI-38) and mouse embryo-fibroblast (NIH/3T3) cell line as standards. Following a 24 h incubation period, the text compounds were added at concentrations ranging from 0.0032 to 32 microg/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 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.

3. Results and Discussion

3.1. Yield and Chain Length

Titanocene polyethers were formed in moderate yield with yield generally decreasing as the PEG chain size increases probably due to the increasing difficulty of the titanocene-containing moieties (both titanocene dichloride and growing end titanocene-containing units) locating and reacting with the PEG end group (Table 1). The products are moderate to high polymers again with chain length decreasing as the PEG chain length increases again possibly related to the increasing difficulty of the metallocene locating the PEG end group.

Table 1. Product yield, molecular weight and chain length as a function of PEG

PEG Mol. Wt./DP	Percentage Yield	Molecular Weight/Water	Molecular Weight/DMSO	Chain Length (DP)
200/4.5	68		5.8×10^6	15000
400/9	42		1.0×10^6	850
1000/27	51	1.8×10^5	1.9×10^5	150
1500/34	52	3.6×10^4	3.7×10^4	21
2000/45	49	3.4×10^4	3.5×10^4	17
3400/77	46	3.7×10^4	3.7×10^4	11
8000/180	41	3.4×10^4	3.4×10^4	4

Water solubility begins when there is sufficient PEG content to allow this to occur, here with the PEG of about 27 units in length. The chain lengths are similar for the water soluble and DMSO soluble systems consistent with lack of chain scission allowing water solubility to occur.

The products are red-brown to tan colored with coloration decreasing as the PEG content increases as expected.

3.2. Thermal Analysis

The polymers from most of the PEGs were solid but those from PEG 1000 and 1500 were tacky solids. The reason(s) for this is unknown. The softening point was measured employing a Fisher-Johns Melting Point Apparatus. The products derived from PEG 200 to 800 did not melt to 300°C. Polymers derived from the higher chain length PEGs did soften over a range of 28 to 55°C (Table 2). It is of interest to note that softening corresponded to the onset of water solubility.

Table 2. Appearance and softening point for the titanocene poly(ethylene glycols) as a function of size of PEG.

PEG	Physical Appearance	Softening Point, C
200	Solid	>300
400	Solid	>300
800	Solid	>300
1000	Tacky Solid	28

1500	Waxy Solid	36
2000	Solid	39
3400	Solid	49
8000	Solid	55

Thermogravimetric analysis was carried out on the products. Figure 3 contains results for the titanocene-PEG 2000 sample. Briefly, in both air and nitrogen weight retention to 600°C varied with the PEG. For the PEG 2000 the percentage weight retention is about 20%. If the residue is titanium alone it would account for 2.5% weight retention and if it were titanium IV oxide it would be about 4% so clearly the residue contains more than the metal or metal oxide. Typical of most metal-containing polymers, the degradation pathways in air and nitrogen are different (1). Weight loss begins about 200°C in air. Softening for the PEG 2000 sample occurs at 39°C (Table 2) so that softening occurs without weight loss.

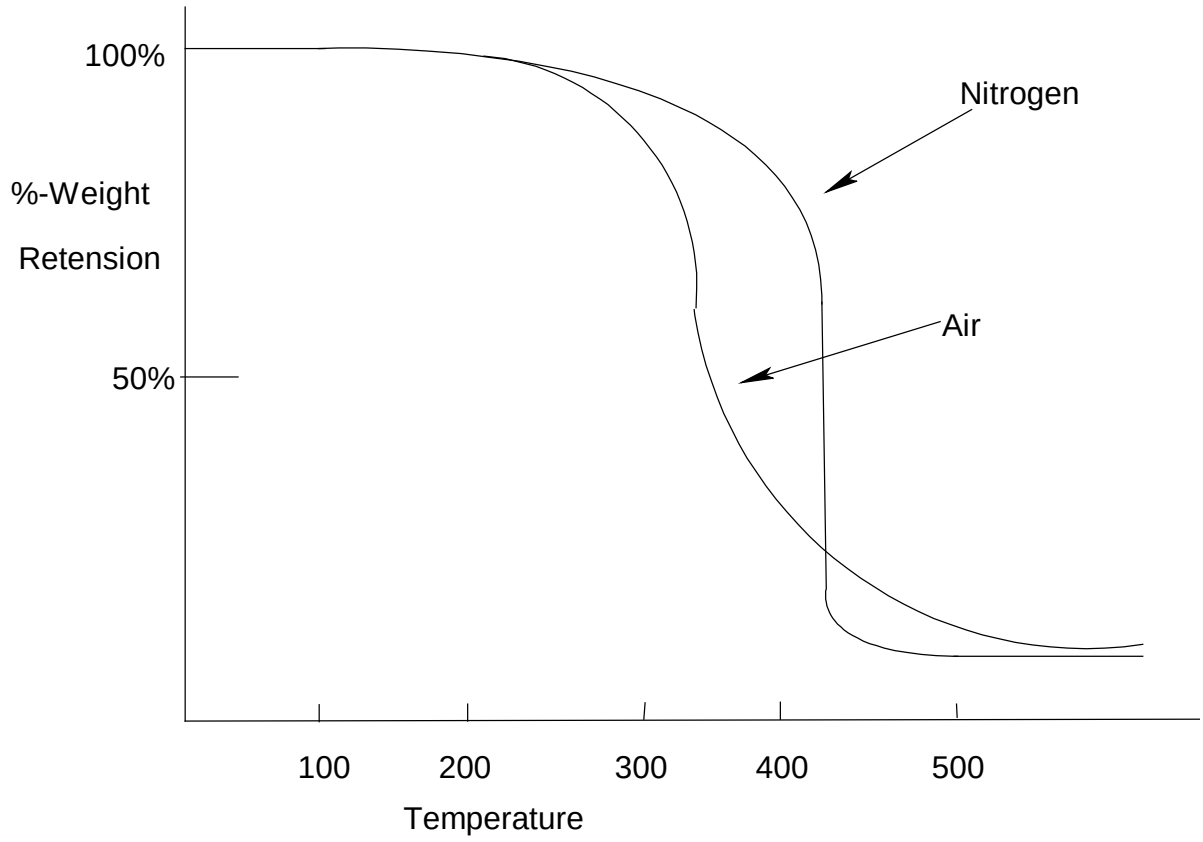


Figure 3. TGA of the titanocene-PEG 2000 sample.

3.3. Elemental Analysis

While the procedure is somewhat rough, it is suitable to give general results. For the product from PEG 2000, the calculated percentage Ti should be 2.3%: found was 2.9%; and for the PEG 3400 the calculated percentage Ti is 1.4 and found was 1.6%.

3.4. Infrared Spectral Results

Infrared spectra were taken of the reactants and products over the range of 4000 to 400 cm^{-1}. All bands are given in cm^{-1}. Band assignments are given in Table 3 for the PEG 200, titanocene dichloride and the polymer derived from reaction between the titanocene dichloride and PEG

200. Briefly, the C-H aromatic stretch for the C-H in the cyclopentadiene moiety is found at 3105 for the product. Bands from the PEG derived moiety assigned to the C-H asymmetric and symmetric stretching are found in the polymer about 2915, 2900, 2885, and 2869. Additional bands assigned to wagging, scissoring, and rocking are also found as are a number of combination bands (Table 3).

Bands assigned to the Ti-O stretch in metallocene polyethers are generally assigned to about 440 for the asymmetric stretch and 345 for the symmetrical stretch. The higher band is found at about 445. The second band is below the capability of the instrument employed in the present study. An additional band attributed to the Ti-O-C unit is assigned to be about 1100. In this area there are two bands, one at 1105 is assigned to the O-C stretch in the PEG and a second new band about 1092 is tentatively assigned to the Ti-O-C formation. Thus vibration spectroscopy is consistent with the assigned repeat unit.

Table 3. Infrared band assignments for titanocene dichloride, PEG 200, and the product.

Band Assignment	Cp_2TiCl_2	PEG 200	Polymer
C-H St	3103		3105
C-H Asym St		2920	2915
C-H Sym St		2890,2885,2865	2900,2885,2869
CH_2 Scissor		1470,1463,1453	1471,1462,1453

Cp(C-C St)	1440		1441
CH_2 Wag		1406	1401
C-C St	1371		1374
CH_2 Twist		1283,1244	1291,1247
C-O St, CH_2 Rock		1149	1150
C-O St		1102	1105
C-C St,C-O St, CH_2 Rock		1062	1063
CH ip Wag	1014		1015
CH_2 Rock,CH_2 Twist		963	961
CH_2 Rock, C-C St		947	943
CH_2 Rock, C-C, C-O St		887	889
C-H op St	873,827		881,828

3.5. MALDI MS

Mass spectra were obtained for the samples. Here we will present results for the PEG 200 product. Masses are described in terms of m/e = 1 or daltons. The assignment of bands is not as straightforward as with many compounds since the PEG is a mixture of chain lengths. For PEG 200, the two most common PEG chains have four (176 daltons) and five (220 daltons) ethylene oxide units with the combination having an average mass of about 200 daltons. Recently we have been employing graphite as the matrix material because we found that it gives good results with few interfering ion fragments produced above 500 mass which is the typical lower mass range employed in our studies.

Tentative assignments are made for the lower mass units and then assignments based on the average chain length of the PEG. Spectra were obtained using two modes, the reflective which is employed favoring precision of results and the linear mode favoring detection of higher mass ion fragments. For the current study we describe the mass range of 500 to 5000 for the reflective mode (Figure 4 and Table 4) and 500 to 2500 (Table 4) for the linear mode. Several abbreviations are employed in describing tentative assignments for the ion fragments. These are U for one average repeat units composed of one Cp_2Ti and an average of 200 dalton for the ethylene oxide moiety; 2U for two average repeat units; 3U for three average repeat units; C5 for five ethylene oxide units; C4 for four ethylene oxide units; C3 for three ethylene units. Sodium is a common contaminant. For fragments greater than 2000 general chain lengths are simply based on taking the fragment values and dividing by 378 the repeat unit based on a PEG of about 200 daltons.

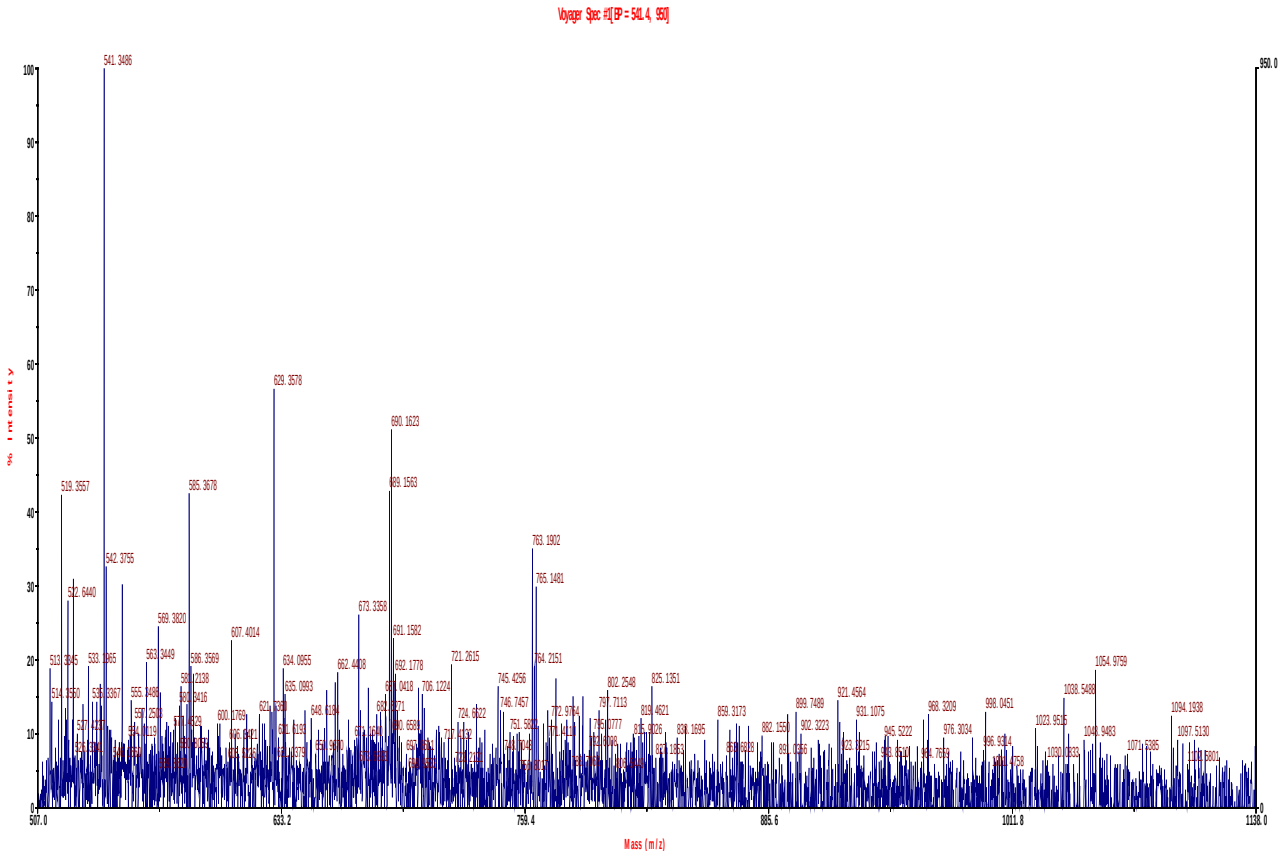


Figure 4. MALDI MS for the product of titanocene dichloride and PEG 200 using the reflective mode with graphite as the matrix over the range of about 500 to 1100.

Table 4. Major ion fragments obtained for the product of PEG200 and titanocene dichloride.

(Tentative) Assignment	Ion fragments the Reflective Mode	Ion fragments for the Linear Mode
C5+Cp_2Ti+C4-2O	541	541
C5+Cp_2Ti+C5-20	585	585
C5+Cp_2Ti+C5-2O+Na	629	629
C4+Cp_2Ti+C4+Cp_2Ti-O	690	689

C4+Cp_2Ti+C4+Cp_2Ti-O	722	723
C4+Cp_2Ti+C5+Cp_2Ti-O+Na	763	763
C5+Cp_2Ti+C5	796	796
C5+Cp_2Ti+C5-O+Na	804	804
C4+Cp_2Ti+C4+Cp_2Ti+C4	877	877
C4+Cp_2Ti+C4+Cp_2Ti+C5-20		897
C4+Cp_2Ti+C4+Cp_2Ti+C5-O	911	910
C5+Cp_2Ti+C5+Cp_2Ti+C5-O+Na	1024	1026
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti	1055	1058
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+Na	1088	1087
C4+Cp_2Ti+C4+Cp_2Ti+C5+Cp_2Ti		1108
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+C4+O		1256
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+C5	1293	1290
C4+Cp_2Ti+C4+Cp_2Ti+C5+Cp_2Ti+C5+O		1345
C4+Cp_2Ti+C5+Cp_2Ti+C5+Cp_2Ti+C5+O		1429
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+O		1475
C4+Cp_2Ti+C4+Cp_2Ti+C4+Cp_2Ti+C4+ Cp_2Ti+C4-O	1612	1613
C4+Cp_2Ti+C4+Cp_2Ti+C5+Cp_2Ti+C5+ Cp_2Ti+C5+Cp_2Ti+C4-0		1803
C4+Cp_2Ti+C4+Cp_2Ti+C5+Cp_2Ti+C5+ Cp_2Ti+C5+Cp_2Ti+C5-O		1837

C4+Cp_2Ti+C5+Cp_2Ti+C4+Cp_2Ti+C5+ Cp_2Ti+C5+Cp_2Ti+C5+Na		1926
6U		2215
6U		2230
6U		2350
6.5U	2449	2449
6.5U	2458	
7U	2680	
8U	2955	
8U	3120	
9.5U	3589	
10U	3893	
11U	4077	
11U	4318	
12U	4627	

It appears that the PEG moiety remains intact and that chain scission occurs with breakage about the Cp_2Ti moiety. Chain lengths to 12 repeat units are found. The particular order that the C4 and C5 moieties appear in the table is not to be considered significant.

Titanium has 5 isotopes. Thus, isotope abundance matches are possible. Tables 5 and 6 contain isotopic abundance matches for two fragment clusters containing one and two titanocene

moieties. The matches are reasonable consistent with the presence of two and three titanocene units within these ion fragments.

Table 5. Isotopic abundance matches for two ion fragment clusters containing a single titanium atom.

Known for Ti		C5+Cp_2Ti+C5-2O		C5+Cp_2Ti+C5-2O	
m/e	Rel. Abund.	m/e	Rel. Abund. Found	m/e	Rel. Abund. Found
46	11	583	10	539	11
47	11	584	11	540	11
48	100	585	100	541	100
49	8	586	8	542	7
50	7	587	6	543	7

Table 6. Isotopic abundance matches for two ion fragment clusters containing two titanium atoms where the relative abundances are greater than 5%.

Known for 2 Ti		C4+Cp_2Ti+C4+Cp_2Ti-O		C4+Cp_2Ti+C4+Cp_2Ti+C4	
m/e	Rel. Abund.	m/e	Rel. Abund. Found	m/e	Rel. Abund. Found
94	22	688	22	875	23
95	21	689	20	876	21
96	100	690	100	877	100
97	16	691	18	878	20

98	15	692	16	879	18

3.6. Proton NMR

NMR was conducted employing d-6 DMSO for the various products. Here we describe results for the product from PEG 200. The titanium dichloride has one band at about 6.6 ppm. PEG has bands at about 3.5-3.6 ppm. The polymer shows a band about 6.5 ppm from the titanocene moiety (cyclopentadiene unit) and 3.5 to 3.6 ppm assigned to the ethylene units from the PEG-derived moiety. The NMR bands in the polymer and monomers are similar consistent with polymer formation having minimal effect on the NMR. These results are consistent with other studies (32, 33).

3.7. Cancer Cell Line Results

Cancer is the leading cause of death globally. The cell lines employed in the current study are given in Table 7. They represent a broad range of important cancers.

Table 7. Cell lines employed in the current study

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	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. Several names are associated with this concentration. We will use the term effective concentration, EC. 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 (34, 35). We have recently found that the anticancer activity is brought about by the intact polymer and not through polymer degradation (25, 35). 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 (25, 34, 35).

Table 8 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 titanocene dichloride is relatively non-toxic (22, 23, 25, 36-38) as are the PEGs. Even so, the ability of various Group IVB metallocene small molecules to inhibit cancer growth is well established (20, 21, 25, 39-43). As noted before, titanocene dichloride was the first-non-platinum metal-containing compound to undergo clinical trial (20). And as noted before, 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 (20, 21).

Table 8. EC_{50} values (micrograms/mL) for the tested cell lines titanocene polymers and monomer, and cisplatin. Values given in () are the standard deviations.

Compound	**NIH-3T3**	**WI-38**	**PANC-1**	**AsPC-1**
Cp_2TiCl_2	>32	>32	>32	>32
Cp_2Ti/PEG 200	0.70(.6)	0.55(.5)	0.81(.7)	0.73(.6)
Cp_2Ti/PEG 400	0.64(.6)	0.56(.6)	0.74(.8)	0.74(.7)
Cp_2Ti/PEG 800	0.63(.6)	0.60(.5)	0.77(.8)	0.75(.7)
Cp_2Ti/PEG 1000	0.71(.7)	0.61(.6)	0.78((.8)	0.76(.7)
Cp_2Ti/PEG 1500	0.70(.5)	0.55(.6)	0.79(.7)	0.77(.7)
Cp_2Ti/PEG 2000	0.70(.6)	0.61(.5)	0.71(.6)	0.77(.8)
Cp_2Ti/PEG 3400	0.69(.6)	0.62(.6)	0.77(.7)	0.80(.7)
Cp_2Ti/PEG 8000	0.69(.5)	0.60(.6)	0.78(.8)	0.76(.6)
Cisplatin	1.2(.2)	0.015(.01)	0.34(.12)	1.4(.15)

Compound	**PC-3**	**MDA**	**MCF-7**	**HT-29**

Cp_2TiCl_2	>32	>32	>32	>32
Cp_2Ti/PEG 200	0.72(.6)	0.73(0.7)	0.81(.7)	0.77(0.7)
Cp_2Ti/PEG 400	0.73(.7)	0.76(.8)	0.79(.8)	0.74(.6)
Cp_2Ti/PEG 800	0.75(.7)	0.79(.8)	0.81(.6)	0.78(.6)
Cp_2Ti/PEG 1000	0.76(.7)	0.74(.7)	.79(.8)	0.77(.7)
Cp_2Ti/PEG 1500	0.78(.7)	0.71(6)	0.75(.8)	0.74(.8)
Cp_2Ti/PEG 2000	0.77(.8)	0.73(.6)	0.80(.7)	0.75(.7)
Cp_2Ti/PEG 3400	0.77(.6)	0.71(.7)	0.82(.8)	0.78(.8)
Cp_2Ti/PEG 8000	0.71(0.7)	0.78(.6)	0.83(.8)	0.80(.7)
Cisplatin	0.001(.0001)	0.001(.0001)	0.003(.0003)	0.002(.0002)

In the United States, about 32,000 individuals are affected with pancreatic cancer. Worldwide this number is 168,000. 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, 44-47). More recently, we found that Group VA-containing polymers also exhibit some inhibition of pancreatic cancer cell lines (48).

The two most widely employed 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. Both are human cell lines and are widely employed in testing for inhibition of pancreatic cancer. As seen in Table 8, while the titanocene dihalide does not inhibit pancreatic cancer cell growth the polymers do at concentration levels comparable to cisplatin itself.

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, 48). 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 one of the most resistant of the prostate cancer cell lines. All 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 (49). 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 titanocene PEG polymers exhibit decent inhibit on the HT-29 cell line.

In general, there appears to be little or no difference in the ability of the polymers to inhibit cancer cell growth as the length of the PEG is varied. Thus, the ability to inhibit cell growth appears to be due to the presence of the titanocene moiety but the presence of the PEG portion is also critical to this inhibition since its presence allows the polymer to have an increased ability to inhibit the various cancer cell lines.

Another measure of the potential use of compounds to inhibit cancer cell growth is the comparison of the ratio of the EC_{50} for the NIH/3T3 (or simply 3T3) or WI-38 cells divided by the EC_{50} for the particular test cell. This value is one of a group called a chemotherapeutic index, CI_{50}.

The CI_{50} values for polymers are given in Table 9. Superimposed in this data is a second study. Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are the NIH 3T3 and WI-38 cell lines. We have begun comparing these two cell lines as biomarkers to study the effectiveness of compounds to inhibit the growth of various tumor cell lines. NIH 3T3 cells are mouse embryo

fibroblast cells. They are part of a group of cell lines referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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.When there is a difference, the WI-38 cell line results are accepted as being more predictive of live-animal results so greater confidence is given to their results (50).

In the current study, the polymer results give similar CI_{50} values using WI-38 and NIH 3T3 cells as the standard so the use of either cell standard is acceptable. But, for the standard cisplatin, this is not the case where there are large differences. Thus, for the PANC-1 cell line, a CI_{50} of 3.5 is calculated for the 3T3 cells but for the WI-38 standard cell line a CI_{50} value of only 0.044 or a difference of about 100. As a quick guide, one can simply look at the ratio of EC_{50} 3T3/EC_{50} WI-38 (or its inverse), so that when this ratio is near one there will be little difference in the CI_{50} values calculated using either cell as standard.

Table 9. CI_{50} values determined from data given in Table 8.

Compound	**EC_{50}3T3/ EC_{50}WI-38**	**EC_{50}WI-38/ EC_{50}3T3**	**EC_{50}3T3/ EC_{50}PANC-1**	**EC_{50}WI-38/ EC_{50}PANC-1**
Cp_2Ti/PEG 200	0.96	0.75	0.97	0.76
Cp_2Ti/PEG 400	0.86	0.76	0.88	0.79
Cp_2Ti/PEG 800	0.84	0.80	0.84	0.80
Cp_2Ti/PEG 1000	0.93	0.80	0.93	0.80
Cp_2Ti/PEG 1500	0.70	0.71	0.90	0.71
Cp_2Ti/PEG 2000	0.91	0.79	0.91	0.79
Cp_2Ti/PEG 3400	0.86	0.78	0.90	0.81
Cp_2Ti/PEG 8000	0.91	0.79	0.97	0.85
Cisplatin	80	0.013	3.5	0.044

Compound	**EC_{50}3T3/ EC_{50}AsPC-1**	**EC_{50}WI-38/ EC_{50}AsPC-1**	**EC_{50}3T3/ EC_{50}PC-3**	**EC_{50}WI-38/ EC_{50}PC-3**
Cp_2Ti/PEG 200	0.96	0.75	0.97	0.76
Cp_2Ti/PEG 400	0.86	0.76	0.88	0.79
Cp_2Ti/PEG 800	0.84	0.80	0.84	0.80

Cp_2Ti/PEG 1000	0.93	0.80	0.93	0.80
Cp_2Ti/PEG 1500	0.91	0.71	0.90	0.71
Cp_2Ti/PEG 2000	0.91	0.79	0.91	0.79
Cp_2Ti/PEG 3400	0.86	0.78	0.90	0.81
Cp_2Ti/PEG 8000	0.91	0.79	0.97	0.85
Cisplatin	0.86	0.01	1200	15

Compound	**EC_{50}3T3/ EC_{50}MDA**	**EC_{50}WI-38/ EC_{50}MDA**	**EC_{50}3T3/ EC_{50}MCF-7**	**EC_{50}WI-38/ EC_{50}MCF-7**
Cp_2Ti/PEG 200	0.96	0.75	0.86	0.68
Cp_2Ti/PEG 400	0.84	0.74	0.81	0.71
Cp_2Ti/PEG 800	0.80	0.76	0.78	0.74
Cp_2Ti/PEG 1000	0.96	0.82	0.90	0.77
Cp_2Ti/PEG 1500	0.99	0.77	0.93	0.73
Cp_2Ti/PEG 2000	0.96	0.83	0.88	0.76
Cp_2Ti/PEG 3400	0.97	0.87	0.84	0.76
Cp_2Ti/PEG 8000	0.88	0.77	0.83	0.72
Cisplatin	3000	2.4	400	5

Compound	**EC_{50}3T3/ EC_{50}HT-29**	**EC_{50}WI-38/ EC_{50}HT-29**
Cp_2Ti/PEG 200	0.91	0.71
Cp_2Ti/PEG 400	0.86	0.76

Cp_2Ti/PEG 800	0.81	0.77
Cp_2Ti/PEG 1000	0.92	0.79
Cp_2Ti/PEG 1500	0.95	0.74
Cp_2Ti/PEG 2000	0.93	0.81
Cp_2Ti/PEG 3400	0.88	0.79
Cp_2Ti/PEG 8000	0.86	0.75
Cisplatin	600	7.5

When evaluating CI_{50} results, values greater than two are considered significant. From Table 9 there are no values greater than two for any of the polymers. Thus, based on CI_{50} values, the titanocene PEG polymers show little activity but based on EC_{50} values they all exhibit decent ability to inhibit all of the cancer cell lines including the pancreatic cancer cell lines. Interestingly, there is no agreement as to which measure is the most significant in predicting the real ability to treat cancer within individuals.

Another concern involves the toxicity of the metal-containing moieties. The toxicity of the metal-containing units varies with the precise compounds as well as mode of measurement and test animal. For cisplatin, the LD50's are as follows-Rat oral 25.8 mg/kg; Rat Intravenous 8.0 mg/kg; mouse oral 32.7 mg/kg; and mouse intravenous 11 mg/kg (Pfizer Material Safety Data Sheet). For titanocene dichloride, the LD_{50} values are rat intraperitoneal 25 mg/kg and mouse intravenous 180 mg/kg (TCI America Material Safety Data Sheet). While exact matches are not available, it appears that cisplatin has lower LD_{50} values, is more toxic, than titanocene dichloride by about three fold.

In preclinical studies titanocene dichloride inhibited ovarian cancer cell lines A2780 CP3 that were twenty fold resistant to cisplatin while the cell line was only about two and a half resistant to titanocene dichloride indicating an absence of cross-resistance between the two metal containing drugs (51). This is consistent with the finding for in vivo studies where titanocene dichloride showed much greater ability to inhibit cisplatin resistant human ovarian cancer xenografts compared to cisplatin. Further, titanocene dichloride largely overcame cisplatin resistance for the A2780CP and CH1cisR ovarian cancer cell lines in bcl-2 and p53 transfectants of A2780 cells (52).

A number of studies are consistent with DNA-metallocene interactions, including the presence of titanocene dichloride, zirconocene dichloride, and hafnocene dichloride, being a major determinant in the anticancer activity of these materials (53).

There is a difference between structural dependencies of our compounds and many metallocenes. It was concluded by some studies that the metallocene dichlorides themselves required liability of the chlorides for activity but our compounds exhibit their activity as polymers rather than control delivery of small units (54-56). Other studies have demonstrated anticancer activity when the halides are replaced so these conclusions are premature (56).

Titanocene dichloride underwent Phase I clinical trials. The trials indicated a dose-limiting side effect associated with nephrotoxicity and a number of unwanted physical side effects including nausea, reversible metallic taste, and pain during infusion, hypoglycemia, with these features undesirable. Counter, the absence of an effect on proliferative activity of the bone marrow, generally a dose-limiting side effect, was positive. Some phase II clinical trials were undertaken with patients with breast metastatic carcinoma (57) and advanced renal cell carcinoma (58). Unfortunately, low activity discouraged further clinical study.

4. Conclusions

The initial synthesis of DMSO and water-soluble titanocene polyethers through reaction of the titanocene dichloride and various poly(ethylene glycols) employing commercially available reactants is described. Infrared, proton NMR, and MALDI MS results are consistent with the titanocene-containing polyether structure. The materials are polymeric with chain lengths ranging from 1500 to 4 in decent yields of about 50%. Yield generally decreases and chain length decreases as the length of the PEG chain increases possibly due to the increasing difficulty of the titanocene finding a PEG end group to react with. Bands characteristic of formation of the Ti-O and Ti-O-C are found in the infrared spectra. MALDI MS shows ion fragments to twelve units. Isotopic abundance values for ion fragment cluster containing one and two titanium atoms are reasonable consistent with the presence of titanium. They exhibit decent inhibition of a variety of cancer cell lines including two pancreatic cancer cell lines. The titanocene polyethers show similar ability to inhibit cancer cell growth independent of the PEG chain length.

This is the first of several studies aimed at both increasing the solubility of titanocene-containing polymers and increasing the toxicity of Group IVB metallocene polymers.

Of importance, the polymers can be easily made in the gram to ton amount since the synthesis employs commercially available materials utilizing the interfacial polycondensation system that has been employed commercially to make polycarbonates and aramids.

References

1. Carraher, C. (2005) Organotin polymers, in Macromolecules Containing Metal and Metal-Like Elements, Vol. 4. Group IVA Polymer, Wiley, Hobokin, 2005.
2. Carraher, C., Roner, M. (2014) J. Organometal. Chem., 751, 67-82.
3. Roner, M., Carraher, C., Shahi, K., Barot, G. (2011) Materials, 4, 991-1012.
4. Carraher, C. (2008) J. Polym. Mater., 25, 35-50.
5. Carraher, C. (2005) J. Inorg. Organometal. Polyms., 15, 121-145.
6. Carraher, C. (1971) J Polym. Sci. Part A-1, 9, 3661-2670.
7. Carraher, C., Bajah, S. (1973) Polymer (Br.), 14, 42-44.
8. Battin, A., Carraher, C. (2008) J. Polym. Mater., 25, 23-33.
9. Carraher, C., Battin, A., Roner, M. (2011) J. Funct. Biomater., 2, 18-30.
10. Carraher, C., Battin, A., Roner, M. (2013) J. Inorganomet. Polym., 23, 61-73.
11. Spessard, G. O., Miessler, G. L. (2016) Organometallic Chemistry, 3rd Ed., Oxford University Press, NY.
12. Bochmann, M. (2015) Organometallics and Catalysis, Oxford University Press, NY.

13. Cotton, F. A., Wilkinson, G. (1999) Advanced Inorganic Chemistry, 6th Ed., Interscience, NY.
14. Benitez, J., Guggeri, L., Tomaz I. (2009) J. Inorg. Biochem., 103(10), 1386-1394.
15. Strohfeldt, K., Tacke, M. (2008) Chem. Soc. Rev., 37(6), 1174-1187.
16. Beckhove, P., Oberschmidt, O., Hanauske, A. (2007) Anticancer Drugs, 18(3), 311-315.
17. Harding, M. M., Mokdsi, G. (2000) Curr. Med. Chem., Dec; 7(12), 1289-303.
18. Olszewski, U., Claffey, J., Hogan, M. Tacke, M., Zeillinger, R., Bednarski, P., Hamilton G. (2011) Invest. New Drugs, Aug; 29(4),607-614.
19. Olszewski, U., Hamilton, G. (2010) Anticancer Agents Med. Chem., May; 10(4):302-311.
20. Roat-Malone, R. M. (2007) Bioinorganic Chemistry, 2ed. Wiley, NY, pp. 19-20.
21. Waern, J. B., Harris, H. H., Lai, B., Cai, Z., Harding, M. M., Dillon, C. T. (2005) J. Bio. Inorg. Chem., 10(5), 443-452.
22. Roner, M., Carraher, C., Shahi, K., Ashida, Y., Barot, G. (2009) BMC Cancer, 9, 358-366.
23. Carraher, C., Roner, M., Shahi, K., Ashida, Y., Barot, G. (2007) J. Polym. Mater., 24, 357-369.
24. Roner, M., Shahi, K., Barot, G., Battin, A., Carraher, C. (2009) J. Inorg. Organomet. Polym., 19, 410-414.
25. Carraher, C., Barot, G., Shahi, K., Roner, M. (2013) J. Chin. Adv. Mater. Soc., 1, 294-304.
26. Barot, G., Shahi, K., Roner, M., Carraher, C. (2007) J. Inorg. Organomet. Polym., 17, 595-603.
27. Carraher, C. (2013) Introduction to Polymer Chemistry,3rd Ed. CRC, Boca Raton, FL.
28. Carraher, C. (2014) Polymer Chemistry, 9th Ed. Taylor and Francis, NY.

29. DiPalma, J., Cleveland, M., Mark, V. B., McGowan, J. Herrera, J. (2007) Am. J. Gastroenterology, 9, 1964-1971.

30. Sheftel, V. O. (2000) Indirect Food Additives and Polymers: Migration and Toxicology, CRC, Boca Raton, FL, pp 1114-1116.

31. Delgado, C., Francis, G. E., Fisher, D. (1992) Drug Carrier Syst., 9, 249-304.

32. Carraher, C., Kloss, J. (1991) Polym. Mater. Sci. Eng., 64, 229-230.

33. Carraher, C., Morrison, A., Roner, M., Moric-Johnson, A., Al-Huniti, M., Miller,L. (2015) J. Chin. Adv. Mater. Soc., 3, 310-327.

34. Carraher, C. (2005) J. Inorg. Organomet. Polym., 15, 121-145.

35. Carraher, C., Barot, G., Vetter, S. W., Nayak, G., Roner, M. (2013). J. Chin. Adv. Mater. Soc., 1, 1-6.

36. Siegmann-Louda, D., Carraher, C., Pflueger, F., Ross, J. R. (2002) Functional Condensation Polymers. Plenum, NY, Chapter 14.

37. Doucette, R., Siegmann-Louda, D., Carraher, C., Cardoso, A. (2004) Polym. Mater. Sci. Eng., 91, 564-566.

38. Carraher, C., Venable, W., Blaxall, H. S., Sheats, J. E. (1980) . J. Macromol. Sci.-Chem., A14, 571-579.

39. Gross, A., Alborzinia, H., Piantavigna, S., Martin, L. L., Wölfl, S., Metzler-Nolte, N. (2015) Metallomics, 7(2), 371-384.

40. Gross, A., Alborzinia, H., Piantavigna, S., Martin, L. L., Wölfl, S., Metzler-Nolte, N. (2014) Chem. Med. Chem., 9(6), 1188-1194.

41. Havelek, R., Siman, P., Cmielova, J., Stoklasova, A., Vavrova, J., Vinklarek, J., Knizek, J., Rezacova, M. (2012) Med. Chem., 8(4), 615-21.

42. Gómez-Ruiz, S, Maksimović-Ivanić, D., Mijatović, S., Kaluđerović, G. N. (2012) Bioinorg. Chem. Appl., 140, 209-218

43. Senthilnathan , D., Vaideeswaran, S., Venuvanalingam, P., and Frenking. G. J. (2011) Mol. Model., Mar. 17(3): 465-475.

44. Barot, G., Roner, M., Naoshima, Y., Nagao, K. Shahi, K. Carraher, C. (2009) J. Inorg. Organomet. Polym., 19, 12-27.

45. Carraher, C. E., Morrison, A., Roner, M. R., Moric, A., Trang, N. (2014) J. Inorg. Organomet. Polym., , 24, 182-189.

46. Roner, M. R., Shahi, K., Barot, G., Battin, A., Carraher. C. E. (2009) J. Inorg. Organomet Polym., 19, 410-414.

47. Carraher, C., Ayoub, M., Roner, M., Moric, A., Trang, N. (2013) J. Chin. Adv. Mater. Soc., 1, 65-73.

48. Carraher, C., Truong, N., Roner, M. (2013) J. Chin. Adv. Mater. Soc., 1, 134-150.

49. Carraher, C., Roner, M., K. Shahi, K., Moric-Johnson, A., Miller, L., Barot, G., Battin, A., Trang, N., Sookdeo, N., Islam, Z. (2015) Int. J. Polym. Mat. Polym. Biomat., 64, 800-814.

50. Ekwall, B., Silano, V., Paganuzzi-stammati, A., Zucco, F. (1990). Toxicity tests with mammalian cell cultures in short-term toxicity tests for non-genotoxic effects. Wiley, NY.

51. Harstrick, A., Schmoll, H., Sass, G., Poliwoda, H., Rustum, Y, Eur. J. Cancer, 1993, 29A7, 1000-1002.

52. Christodoulou, C. V., Eliopoulos, A. G., Young, L. S., Hodgkins, L, Ferry, D. R., Kerr, D. J., (1998) Br. J. Cancer, 77(12), 2088-2097.

53. Murray, J. H., Harding, M. M. (1994) J. Med. Chem., 37, 1936-1941.

54. Harding, M. M., Mokdsi, G., (2000) Curr. Med. Chem., 7, 1289-1303.

55. Guang-Xiang, L, Wang, M-L., Su, Li-Jun, Zou, Y., Wu, Y-Q, (2011) Wuji Huaxue Xuebao, 27, 1185-1187.

56. Abeysinghe, P. M., Harding, M. M. (2007) Dalton Trans., 3474-3482.

57. Kroger, N., Kleeberg, U. R., Mross, K., Edler, L., Sab, G., Hossfeld, K. (2000) Onkologie, 23, 60-62.

58. Lummen, G., Sperling, H., Luboldt, H., Otto, T., Rubbe, H. (1998) Cancer Chemotherapy Pharmacology, 42(5), 415-417.

CHAPTER 12

METALLOCENE-CONTAINING POLYESTER FROM REACTION OF 3,5-PYRIDINEDICARBOXYLIC ACID AND METALLOCENE DIHALIDES AND THEIR PRELIMINARY ABILITY TO INHIBIT CANCER CELL GROWTH[10]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 12 and 13) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher Jr.,C.E.; Morrison, A.; Roner, M.R.; Moric-Johnson, A.; Al-huniti, M. H; and **Miller, L**.

2015.

Carraher Jr.,C.E.; Morrison, A.; Roner, M.R.; Moric-Johnson, A.; Al-huniti, M. H; and **Miller, L**. **2015**. Metallocene-Containing Polyesters from Reaction of 3,5-Pyridinedicarboxylic Acid and Metallocene Dihalides and Their Preliminary Ability to Inhibit Cancer Cell Growth. Journal of the Chinese Advanced Materials Society. DOI: 10.1080/22243682.2015.1072732.

[10] Used with permission of Taylor & Francis, 2017

Metallocene-Containing Polyesters from Reaction of 3,5-Pyridinedicarboxylic Acid and Metallocene Dihalides and Their Preliminary Ability to Inhibit Cancer Cell Growth

Charles E. Carraher, Jr. ,[a] Alicia Morrison,[a] Michael R. Roner[b], Alisa Moric-Johnson,[b] Mohammed H. Al-Huniti,[a] and Lindsey Miller,[b]

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 (carraher@fau.edu**)**

b. University of Texas Arlington, Department of Biology, Arlington, TX 76010 (roner@uta.edu)

*Corresponding author: Charles E. Carraher, Jr.; Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431; carraher@fau.edu; 561-297-2107; fax 561-297-2759; words approximately 7,500.

Abstract: Polyesters were rapidly formed employing the interfacial condensation of Group VB and vanadocene dihalides with 3,5-purinedicarboxylic acid. Infrared spectroscopy shows the formation of metal-oxygen bonds. The products have a largely distorted non-bridging geometry about the metal atom. MALDI MS results are consistent with the formation of the polyesters. The polymers exhibit inhibition of the growth of pancreatic, colon, breast and prostate cancer cell lines.

Keywords: Group VA metallocene polymers; titanocene polymers; zirconocene polymers; hafnocene polymers; vanadocene polymers; 3,5-pyridinedicarboxylic acid; interfacial polymerization; pancreatic cancer; breast cancer; colon cancer; prostate cancer

1. Introduction

We have been engaged in the synthesis of a variety of polymers including metal-containing polymers for a variety of purposes. Included in the metal atoms incorporated into polymers are tin [1-18], Group IVB metallocenes [19-31], platinum [32,33] and Group VA metals, namely arsenic, antimony, and bismuth [34-42]. Much of the most recent effort has focused on the special properties that can be incorporated into such polymers because of the presence of the metal-moiety. One area of effort is the synthesis of polymers that can offer value as anticancer, antiviral, and antibacterial materials. Some of this effort has involved employing biologically active agents as the Lewis base with the idea that this biological activity might impart particular biological characteristics to the polymer that might enhance the ability of the products to inhibit unwanted growth in bacteria, viruses, and cancers.

3,5-Pyridinedicarboxylic acid, PA, also known as 5-carboxynicotinic acid, dinicotinic acid, and 3,5-pyridinedicarboxate (CAS 499-81-0) was reported to be a competitive inhibitor of bovine liver glutamate dehydrogenase [43,44]. Thus, it fits our approach of employing biologically active Lewis bases coupled with biologically active metal-containing Lewis acids.

There have been several reports describing the synthesis of polymers from 3,5-pyridinedicarboxylic acid, PA. Many utilize the diester of PA rather than direct polymer synthesis employing PA. Ogata described the synthesis of various polyamides and polyesters from the reaction of the diesters with various diamines and diacid chlorides [45]. Marvel and Vogel reported the formation of polybenzimidazoles through reaction of the phenyl ester [46]. Hopff and Krieger described the synthesis of polyamides though heating of the diamine salt with the ester of PDA [47]. Shizunobu and Yashiko [48] synthesized polyesters from the reaction of 3,5-pyridinedicarboxylic acid with various aromatic and aliphatic acid dichlorides employing the melt and interfacial processes. Braz and coworkers [49] prepared polyoxamides from the reaction of 3,5-pyridinedicarboxylic acid with various acid dichlorides. Hergenrother, Wrasidlo, and Levine [50] synthesized polybenzothiazoles from the condensation of aromatic bis(mercaptoamines) and aromatic dicarboxylic acids including 3,5-pyridinedicarboxylic acid.

A number of organotin-products have been synthesized that included PA. Pellerito and coworkers synthesized a number of organotin pyridine-containing complexes using solution reactions including those from PA. The organotin reactants were generally hydroxyl-containing reactants such as Ph_3SnOH [51,22]. Ma and coworkers synthesized a number of polymeric ionic triorganotin esters of PA through refluxing solutions containing the reactants. The products are complex two-dimensional arrays [53].

A number of coordination polymers have also been formed employing 3,5-pyridinedicarboxylic acid. Qison, Benfeng, Qiuyan and Taiqi [54] and Qisong and Faqiang [55] formed coordination polymers from reaction of PA with terbium salts. The products exhibited green light-emitting qualities as well as strong fluorescence bands. Chandrasekhar and Thirumoorthi formed a number of coordination macrocycles from reaction of organotin chlorides and oxides with PA [56]. Other polymeric structures have been reported from reaction with a variety of metal ions. A collection of these are cited [57-65].

Can either add or substitute for references 57-65.

H. Fei, X. Liu, Z.Li, W. Feng, Metal dicarboxylates: new anode materials for lithium-ion batteries with good cycling performance, Dalton Trans. 2015, 44, 9909-9914.

S. Alisir, S. Demir, B. Sariboga, O. Buyukgungor, A disparate 3-D silver(I) coordination polymer of pyridine-3,5-dicarboxylate and pyrimidine with strong intermetallic interactins: X-ray crystaliography, photoluminescane and antimicrobial activity, J. Coord. Chem., 2015, 68, 155-168.

M. Wang, F. Wang, J. Ma, J. Xu, Organic linker geometry controlled synthesis of coordination polymer spheres and their thermal transformation to yolk-shell metal oxides, J. Materials Chem. A: Materials for Energy and Sustainability, 2014, 2, 15480-15487.

J. Chen, H. Zhao, H. Li, S. Huang, N. Ding, W. Chen, D. Houng, W. Zhang, T. Andy Hor, Cryst. Eng. Comm. 2014, 16, 7722-7730.

Y. Liu, Y. Du, X. Wu, Z. Zheng, X. Lin, L. Zhu, Y. Cai, A series of lanthanide complexes based on pyridine-3,5-dicarboxylate and succinate ligands: syntheses, structures, and properties, Cryst. Eng. Comm. 2014, 16, 6797-6802.

J. Chen, M. Chen, N. Ding, W. Chen, H. wen-Hua, T. S. Andy, D. J. Young, Transmetalation of a dodecahedral Na9 aggregate-based polymer: a facile route to water stable Cu(II) coordination networks, Inorg. Chem., 2014, 53, 7446-7454.

K. Liu, H. Liu, L. Yang, F. Zho, Y. Li, W. Ruan, One-pot synthesis of monodisperse Zn coordination polymer micro/nanostructures and their transformation to mesoporours ZnO photocatalysts, RSC Advances 2014, 4, 25160-25164.

G. Nanai, R. Thankuria, H. Titi, R. Patra, I. Goldberg, Synthesis, structure, topology and magnetic properties for new coordination polymers based on 5(-Br/-COOH substituted nicotinic acid, Cryst. Eng. Comm., 2014, 16, 5244-5256.

While the term metallocenes covers a wide variety of materials we will employ it to describe the Group IVB metallocenes and vadancene and even here only the dihalides. These metallocenes are distorted tetrahedral in geometry. While they have been employed in the formation of many materials, their largest use is as soluble stereoregular catalysts allowing the synthesis of a wide variety of stereoregular polymers including polyethylenes and polypropylenes [66,67]. Cotton and Wilkinson [68] describe that Group IVB metallocenes, and vanadocene dichloride, can be considered 9-coordinate bonding species (the hybrid orbitals being derived from one-s, three-p, and five-d orbitals) . Each pi-Cp ring involves three hybrid orbitals. The three remaining orbitals consist of two equivalent spd(x^2-y^2), dz^2 orbitals (overlapping with the two chloride atoms) and one sp orbital which is vacant and believed to be responsible for the catalytic activity of the Group IVB metallocenes [66-69].

The topic of metallocene polymers has been recently reviewed [31]. As noted before [19-31], we have been involved in the inclusion of metallocenes into polymer backbones for a number of reasons including most recently the production of electrically conductive materials

[28, 70,71] and their ability to inhibit cancer [30,39]. The ability to inhibit cancer cell growth is well known and the mechanistic picture has been recently reviewed and found to be different than for cisplatin[72-77]. Having the mechanism different than that for cisplatin is an advantage since it allows the metallocenes to be part of a "mixture of drugs" delivered to patients that will intersect cancer growth at different sites.

We recently reported the synthesis of organotin polymers derived from the synthesis of organotin dihalides and 3,5-pyridinedicarboxylic acid (Figure 1, right) [78]. Here we report the synthesis of the analogous metallocene polyesters (Figure 1, left).

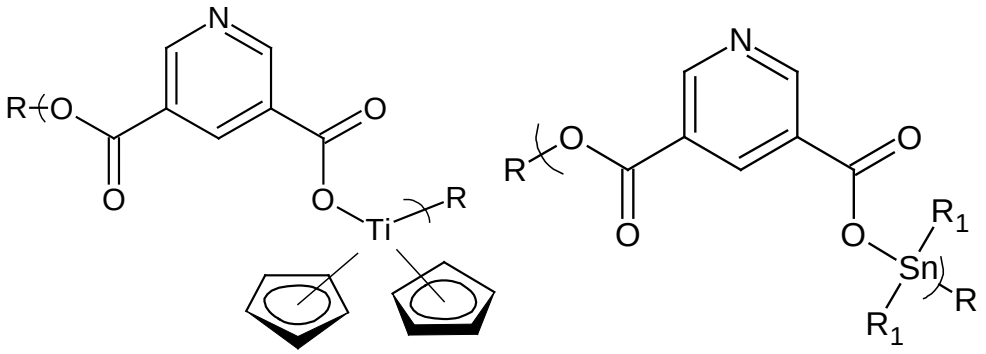


Figure 1. Repeat unit for the products of 3,5-pyridinedicarboxylic acid and titanocene dichloride (left) and organotin dihalides (right).

2. Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the PA (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 ^{o}C). Stirring was begun and a chloroform solution (30 ml) containing the metallocene dihalide (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 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), PA (499-81-0) and zirconocene dichloride (1291-32-3) were purchased from Aldrich Chemical Co., Milwaukee, WS; and hafnocene dichloride (12116-66-4) and vanadocene dichloride (12083-48-6) were obtained from Ventron Alfa Inorganics, Beverly, Mass. The reactants were used as received.

2.2 Physical Characterization

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 2000 to 100,000. Fifty to two hundred shots were typically taken for each spectrum. Results employing alpha-cyano-4-hydroxycinnamic acid are included in the present paper. The solid product along with solid matrix were mixed together employing copper spheres giving a fine powder that was employed to obtain the spectra.

Light scattering photometry was carried out employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the polymers dissolved in 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.

2.3 Cell Testing

The toxicity of each test compound was evaluated using a variety of cancer cell lines and with human normal embryonic lung fibroblast (WI-38) and mouse embryo-fibroblast (NIH/3T3) cell line as standards. Following a 24 h incubation period, the text compounds were added at concentrations ranging from 0.0032 to 32 microg/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 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.

3. Results and Discussion

3.1. Yield and chain length

Metallocene polyesters were formed in poor (for vanadocene) to good (titanocene, zirconocene and hafnocene) yield. The products are moderate to high polymers. For the Group IVB

metallocenes, both yield and chain length increase as the metal size increases. In terms of the hard/soft concept, this is consistent with yield and chain length increasing as the metal becomes softer. This is also consistent with the formation of other Group IVB metallocene polyesters [31]. The low yield and moderate chain length for the vanadocene product is also similar to other syntheses of vanadocene polymers [31].

Table 1. Product yield, average molecular weight, and chain length for the metallocene polyesters.

Metallocene	Percentage Yield	Molecular Weight	Chain Length, DP
Cp_2Ti	24	3.8×10^5	1100
Cp_2Zr	86	2.0×10^6	4200
Cp_2Hf	88	3.3×10^6	7000
Cp_2V	5	1.7×10^5	490

3.2. Vibrational Spectroscopy

Infrared spectral analysis was carried out for all of the samples over the range of 4000-650 cm^{-1}. All band locations are given cm^{-1}.

Infrared spectral analysis is consistent with the proposed structure and with other reported analyses [15,22,27,29-31, 44,51-53,62,69,78]. Table 9 contains results for monomers and polymers. All of the spectra show bands characteristic of both reactants and a new band for the product assigned to the M-O-C(O) linkage (Table 10). Bands assigned to the M-O stretch in metallocene polyethers are generally assigned to about 440 for the asymmetric stretch and 345 for the symmetrical stretch[29]. These two bands are below the capability of the instrument employed in the present study. The asymmetric stretch for the M-O-C(O) group is identified as around 1150 and the corresponding symmetric stretch about 1070 [15, 79]. For the titanium product these bands appear at 1140 and 1076; for the zirconium polymer at 1142 and 1080; for hafnium at 1140 and 1067; and vanadium at 1140 and the second band buried in a broad band around 1060. Bands associated with the C-H stretching are present in the polymers characteristic of the presence of both the Group IVB Cp_2M moiety around 3100 and the PA moiety about 3090. Bands present in PA associated with the presence of the OH moiety are absent in the polymers. The major bands associated with the Cp moiety are present in the polymers. In summary, bands are present that are characteristic of the presence of both moieties in the polymers and the formation of the M-O-C(O) unit.

Table 2. Selected infrared spectral band assignments for titanocene dichloride, PA and the polymers derived from reaction of various metallocene dichlorides with PA.

Assignment	Cp_2TiCl_2	PA	Cp_2Ti/PA	Cp_2Zr/PA	Cp_2Hf/PA	Cp_2V/PA
C-H St (Ar)	3103	3090	3117,3090	3101,3089	3101,3067	3091
C=O St		1640	1626	1642	1640	1640
Ring St		1565	1565	1594	1557	1574
OH ip Wag		1337,1330				
CH ip Wag	1015		1026	1013	1017	1031
Ring Breathing		996	963	980	947	962
CH op Wag		889	890	890	890	899
Skelatal-C Inversion		854	864	840	850	850
CH op Bending	820		828	823	818	823

The geometry about the metal atom can be either what is referred to as bridging or non-bridging (Figure 2). This leads to the product being either a distorted tetrahedral (non-bridging) or distorted octahedral (bridging). Typically the trigonal bipyrimidal structure formed from one bridging and one non-bridging structure is not formed [31].

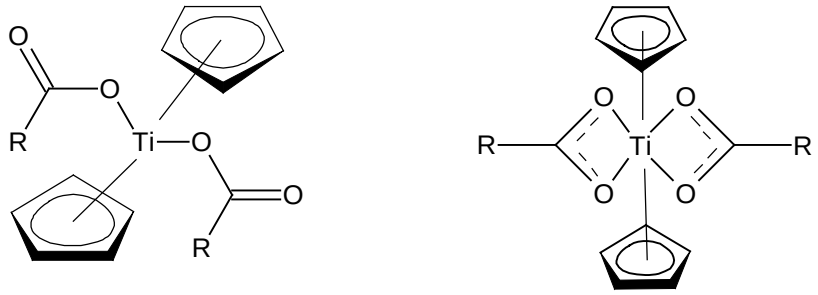


Figure 2. Geometry about the central atom-distorted tetrahedral (left) and distorted octahedral (right) for M = titanium.

Infrared spectroscopy is the easiest way to determine the presence of bridged and non-bridging structures [12,31,34,42,78]. Bridging asymmetric carbonyl absorptions are found around 1545-1570. The bridging symmetric carbonyl band is found around 1410-1430. Non-bridging asymmetric carbonyl bands are found about 1600-1655; and the corresponding symmetric carbonyl bands are found about 1340-1370. Results for the products are given in Table 3.

Table 3. Infrared bands associated with bridging and non-bridging structures.

Metal-Cont. Moiety	Asy. Bridging	Sym. Bridging	Asy. Non-bridging	Sym. Non-bridging
Cp_2Ti	1514vve	1447 vwe	1626 st	1360 st
Cp_2Zr			1642 st	1360 mst
Cp_2Hf	1557 wk	1413 wk	1649 st	1354 st
Cp_2V			1633 st	1350 st

where st = strong; med=medium; we = weak; v = very and NF = not found

The strongest bands are found consistent with non-bridging while bands assigned to bridging are either weaker or not present. The distorted tetrahedral structure is less demanding sterically so the non-bridging is expected because of the presence of the Cp groups and somewhat bulky pyridine moiety.

3.3. MALDI MS

For about a dozen years we and others have been employing MALDI MS for the identification of a number of non-volatile metal and non-metal containing polymers. This has been recently reviewed [12, 31, 80-82]. The technique employed by us is not straight forward MALDI MS but it is applicable to soluble and insoluble products so has wide potential for application. Since this new technique focuses on the fragments that are created in the MALDI MS process, the approach is sometimes referred to as Fragmentation Matrix-Assisted Laser Desorption/ Ionization mass spectrometry or simply F MALDI MS because it is the fragments that are emphasized in the study. The technique should be applicable to any solid when the proper operating conditions are employed.

Figure 3 contains the MALDI MS for the product from titanocene dichloride and PA. There are six major ion fragment clusters derived from the polymer. Table 4 contains tentative assignments for these ion fragment clusters. Several abbreviations are employed when describing the possible assignments: Cp is the cyclopentadiene moiety; U is one unit; and 2U is two units. Sodium is commonly present as a contaminant. Ion fragment clusters to one and a half units are present.

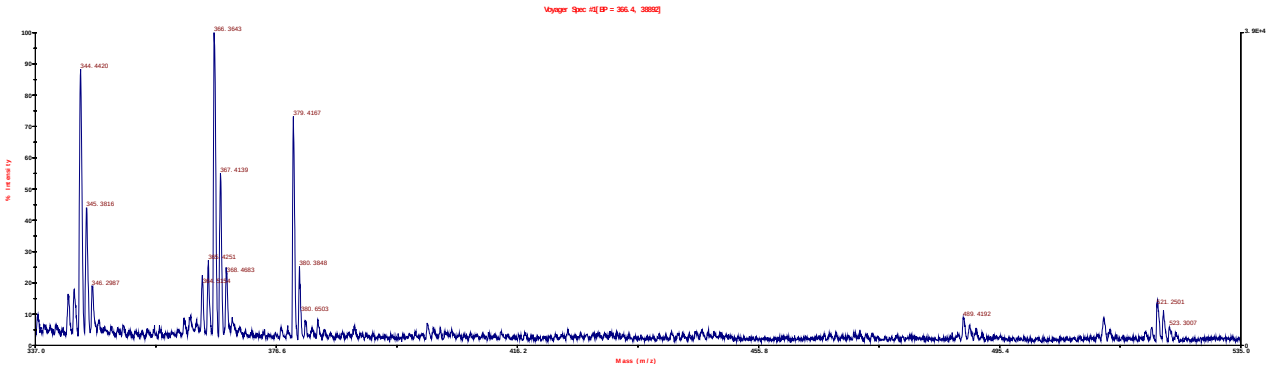


Figure 3. MALDI MS for the product of titanocene dichloride and PA over the range of 350 to 600 Da.

Table 4. Most abundant ion fragment clusters derived from the products of PA and titanocene dichloride from 300 to 800 Da.

Mass (Da)/Assignment		**Mass (Da)/Assignment**	
344	U	489	U+CpTi, 2O
366	U,Na	510	U+PA
379	U,O,Na	521	U+Cp_2Ti

Titanium has 5 isotopes. Thus, isotope abundance matches are possible. Table 5 contains such matches for two ion fragment clusters containing a single titanium atom each. The match is reasonable.

Table 5. Isotopic abundance matches for two ion fragment clusters containing a single titanium atom.

Known for Ti		**U**		**U,Na**	
m/e	Rel. Abund.	m/e	Rel. Abund. Found	m/e	Rel. Abund. Found
46	11	342	12	364	13
47	11	343	12	365	12
48	100	344	100	366	100
49	8	345	10	367	10
50	7	346	9	368	8

Table 6 contains the major ion fragment clusters for the product of PA and zirconocene dichloride. Ion fragments to two and a half units are found.

Table 6. Most abundant ion fragment clusters derived from the products of PA and zirconocene dichloride from 300 to 1000 Da.

Mass (Da)/Assignment		Mass (Da)/Assignment	
342	U-CO_2	640	U+Cp_2Zr,CO_2
408	U,Na	707	2U-Cp
535	U+PA-O	788	2U+O
586	U+CpZr,CO_2	943	2U+Cp_2Zr,O
607	U+Cp_2Zr		

Zirconium has four isotopes present in a relative abundance greater than ten percent. Table 7 contains isotopic abundances for two ion fragment clusters each containing a single zirconium atom. Again, the matches are reasonable consistent with the presence of a single zirconium in these ion fragment clusters.

Table 7.Isotopic abundance matches for two ion fragment clusters containing a single zirconium atom.

Known for Zr		U-CO_2		U,Na	
m/e	Rel. Abund.	m/e	Rel. Abund. Found	m/e	Rel. Abund. Found
90	100	342	100	408	100
91	22	343	30	409	29
92	33	344	36	410	36
94	33	346	33	412	31

Table 8 contains the major ion fragments for the product derived from hafnocene dichloride and PA. Again, ion fragment clusters to two units are found.

Table 8. Most abundant ion fragment clusters derived from the products of PA and hafnocene dichloride from 300 to 1000 Da.

Mass (Da)/Assignment		Mass (Da)/Assignment	
413	U-Cp	656	U+Hf
432	U-Cp+O	687	U+Hf,2O
478	U	833	U+Cp_2Hf,CO_2

498	U+Na	853	U+Cp_2Hf,CO_2,Na
524	U+CO_2	875	U+$Cp_2Hf,2CO_2$,Na
554	U+PA-$2CO_2$	971	2U+O
621	U+PA-CO_2+Na		

Hafnium has five isotopes with relative abundances greater than ten percent. Table 9 contains an isotope match for an ion fragment cluster containing a single hafnium atom and one containing two hafnium atoms. The agreements are reasonable consistant with the ion fragment clusters containing one and two hafnium ions.

Table 9. Major ion fragment clusters for the product of hafnocene dichloride and PA containing one hafnium atom, top, and two hafnium atoms, bottom (relative abundances >10%).

Known for Hf		**U,Na**	
m/e	Rel. Abund.	m/e	Rel. Abund. Found
176	15	494	15
177	53	495	58
178	78	496	78
179	39	497	41
180	100	498	100

Known for 2Hf		**2U+O**	
m/e	Rel. Abund.	m/e	Rel. Abund. Found
354	31	967	32
355	55	968	55
356	77	969	60
357	98	970	98
358	100	971	100
359	46	972	48
360	59	973	52

Table 10 contains the most abundant ion fragments for the product of vanadocene dichloride and PA. Again, ion fragments to two units are found. While vanadium contains isotopes it is essentially 100% isotope 51 so reasonable ion fragment matches are not possible.

Table 10. Most abundant ion fragment clusters derived from the products of PA and vanadocene dichloride from 300 to 800 Da.

Mass (Da)/Assignment		Mass (Da)/Assignment	
347	U	487	U+PA,Na-CO_2
369	U+Na	503	U+PA-O
392	U+CO_2	515	U+PA
417	U+CO_2,Na	527	U+Cp_2V
445	U+PA-2CO_2,Na	656	2U+2O-Cp
467	U+PA-CO_2	695	2U

As in other studies, bond cleveage occurs at the hetroatoms within the chains and the PA ring is left intact consistent with the mildness of MALDI MS.

In summary, MALDI MS results are consistent with the proposed repeat unit and typically contain ion fragment clusters to two units. Isotopic abundance matches are reasonable consistent with the presence of metal ions in these ion fragment clusters.

3.4. Proton NMR spectroscopy

Proton NMR was carried out on the products and monomers. The 3,5-pyridinedicarboxylic acid shows two proton environments associated with the pyridine ring (Figure 3). For 3,5-pyridinedicarboxylic acid itself these bands appear for environment "a" at 7.4 and for "b" at 6.8 (all band locations are given in ppm). For the polymers these bands appear between 7.4-7.5 and 6.8 consistent with the presence of the 3,5-pyridinedicarboxylic moiety. Because the protons in the 3,5-pyridinecarboxylic ring are isolated from the organotin moiety, these bands appear little changed as the substituents on the metal are changed. All three metallocene dichlorides show a doublet at about 6.5. The titanocene polymer shows four bands, a doublet at 6.5 and 6.4 assigned to the cyclopentadiene moiety; bands at 6.7 and 7.5 assigned to the pyridine moiety. The zirconocene shows a doublet at 6.5 and 6.4 from the cyclopentadiene moiety and bands at 6.7 and 7.6. Finally, the hafnocene polymers shows a doublet at 6.5 and 6.5 and bands at 6.7 and 7.5. The solubility of the vanadocene product was too low to allow NMR to be done.

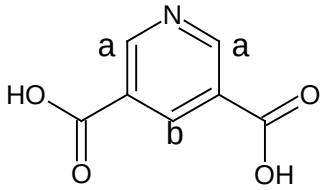


Figure 4 3,5-Pyridinedicarboxylic acid with assigned protons.

Thus, proton nmr spectroscopy results are consistent with the presence of moieties from both reactants being present in the product. Because of the poor solubility of the metallocene polymers in the d6 DMSO little additional information is gained from the proton nmr results.

3.4 Cancer Cell Line Results

Cancer is the leading cause of death globally. The cell lines employed in the current study are given in Table 11. They represent a broad range of important cancers.

Table 11. Cell lines employed in the current study

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	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. Several names are associated with this concentration. We will use the term effective concentration, EC. 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 [31,83]. We have recently found that the anticancer activity is brought about by the intact polymer and not through polymer degradation [83,87]. 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 [31,83,88].

Table 12 contains EC_{50} values for the present compounds and monomers. Cisplatin, among the most widely employed chemo-agent is included as a standard. Consistent with other studies done by us the metallocene monomers are relatively non-toxic [29,30,89,90] as is the Lewis base.

Even so, the ability of various Group IVB metallocene small molecules to inhibit cancer growth is well established (30,39, 72-77). In fact, titanocene dichloride was the first-non-platinum metal-containing compounds to undergo clinical trial (91). 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 (91,92).

In the United States about 32,000 individuals are affected with pancreatic cancer. Worldwide this number is 168,000. 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 [6,78,83-85]. More recently, we found that Group VA-containing polymers also exhibit some inhibition of pancreatic cancer cell lines [86].

The two most widely employed 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. Both are human cell lines and are widely employed in testing for inhibition of pancreatic cancer. 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.

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, coloredtal adenocarcinoma, and bowel cancer. The focus in 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 on the HT-29 cell line.

Table 12. EC_{50} values (micrograms/mL) for the tested cell lines for 3,5-pyridinedicarboxylic acid (PA) and the metalocene-containing monomers and polymers. Values given in () are the standard deviations.

Compound	**NIH-3T3**	**WI-38**	**PANC-1**	**AsPC-1**
PDA	>32	>32	>32	0.830(.025)
Cp_2TiCl_2	>32	>32	>32	>32
Cp_2Ti/PDA	29 (5)	18 (4)	2.1(1)	4.4(2)
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PDA	27(5)	20(4)	11(3)	5.5(3)
Cp_2HfCl_2	>32	>32	>32	>32
Cp_2Hf/PDA	28(5)	20(4)	4.6(3)	3.7(3)
Cp_2VCl_2	>32	>32	>32	>32
Cp_2V/PDA	21(4)	20(4)	2.2(2)	7.5(4)
Cisplatin	1.2(.2)	0.015(.01)	0.34(.12)	1.4(.15)

Compound	**PC-3**	**MDA**	**MCF-7**	**HT-29**
PDA	>32	>32	>32	>32
Cp_2TiCl_2	>32	>32	>32	>32
Cp_2Ti/PDA	5.1 (3)	5.7 (1)	2.3(1)	4.4(1)
Cp_2ZrCl_2	>32	>32	>32	>32
Cp_2Zr/PDA	17(5)	19(5)	14(4)	18(4)
Cp_2HfCl_2	>32	>32	>32	>32
Cp_2Hf/PDA	1.1(1)	4.1(1.5)	4.7(1.5)	2.7(1)
Cp_2VCl_2	>32	>32	>32	>32
Cp_2V/PDA	3.1(2)	6.6(2)	4.0(1)	2.8(1)

Cisplatin	0.001(.0001)	0.001(.0001)	0.003(.0003)	0.002(.0002)

Another measure of the potential use of compounds is the comparison of the ratio of the EC_{50} for the NIH/3T3 (or simply 3T3) or WI-38 cells divided by the EC_{50} for the particular test cell. This value is one of a group called a chemotherapeutic index, CI_{50}.

The CI_{50} values for polymers are given in Table 13. Superimposed in this data is a second study. Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are the NIH 3T3 and WI-38 cell lines. We have begun comparing these two cell lines as biomarkers to study the effectiveness of compounds to inhibit the growth of various tumor cell lines. NIH 3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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. When there is a difference, the WI-38 cell line results are accepted as being more predictive of live-animal results so greater confidence is given to their results (93).

In the current study, the polymer results give similar CI_{50} results so either cell standard is acceptable. But, for the standard cisplatin, this is not the case where there are large differences. Thus, for the PANC-1 cell line, a CI_{50} of 3.5 is calculated for the 3T3 cells but for the WI-38 standard cell line a CI_{50} value of only 0.044 or a difference of about 100. As a quick guide, one can simply look at the ratio of EC_{50} 3T3/EC_{50} WI-38 (or its inverse). When this ratio is near one there will be little difference in the CI_{50} values calculated using either cell as standard.

Table 13. CI_{50} values determined from data given in Table 12.

Compound	**EC_{50}3T3/ EC_{50}WI-38**	**EC_{50}WI-38/ EC_{50}3T3**	**EC_{50}3T3/ EC_{50}PANC-1**	**EC_{50}WI-38/ EC_{50}PANC-1**
Cp_2Ti/PDA	1.6	0.62	**14**	**8.6**
Cp_2Zr/PDA	1.3	0.74	**2.5**	1.8
Cp_2Hf/PDA	1.4	0.71	**6.1**	**4.4**
Cp_2V/PDA	1.1	0.99	**10.**	**9.1**
Cisplatin	**80**	0.013	**3.5**	0.044

Compound	**EC_{50}3T3/ EC_{50}AsPC-1**	**EC_{50}WI-38/ EC_{50}AsPC-1**	**EC_{50}3T3/ EC_{50}PC-3**	**EC_{50}WI-38/ EC_{50}PC-3**
Cp_2Ti/PDA	**6.6**	**4.1**	**5.7**	**3.5**
Cp_2Zr/PDA	**4.9**	**3.6**	1.6	1.2
Cp_2Hf/PDA	**7.6**	**5.4**	**25**	**18**
Cp_2V/PDA	**2.8**	**2.7**	**6.8**	**6.5**
Cisplatin	0.86	0.01	**1200**	**15**

Compound	**EC_{50}3T3/ EC_{50}MDA**	**EC_{50}WI-38/ EC_{50}MDA**	**EC_{50}3T3/ EC_{50}MCF-7**	**EC_{50}WI-38/ EC_{50}MCF-7**
Cp_2Ti/PDA	**5.1**	**3.2**	**13**	**7.8**
Cp_2Zr/PDA	1.4	1.1	1.9	1.4
Cp_2Hf/PDA	**6.8**	**4.9**	**6.0**	**4.3**
Cp_2V/PDA	**3.2**	**3.0**	**5.3**	**5.0**
Cisplatin	**3000**	**2.4**	**400**	**5**

Compound	**EC_{50}3T3/ EC_{50}HT-29**	**EC_{50}WI-38/ EC_{50}HT-29**
Cp_2Ti/PDA	**6.4**	**4.1**
Cp_2Zr/PDA	1.5	1.2
Cp_2Hf/PDA	**10**	**7.4**
Cp_2V/PDA	**7.5**	**7.1**
Cisplatin	**600**	**7.5**

When evaluating CI_{50} results, values greater than two are considered significant. From Table 13 there are values greater than two for most of the cell lines for all three of the metallocene polymers. Values of two and greater are given in bold for easy identification. The vanadocene polymer showed values greater than two for all of the cancer cell lines. For the two pancreatic cancer cell lines, values range from 1.8 to 14 with titanocene, hafnocene and vanadocene polymers offering the larger values and merit further testing. The values for the two breast cancer cell lines are similar again with the titanocene, hafnocene, and vanadocene polymers offering the higher values with values ranging from a low of 3.2 to 13. For the prostate PC-3 cell line, values are again high for the titanocene, hafnocene and vanadocene polymers with the hafnocene values being 18 and 25. For the colon cell line again the higher values are also found for the titanocene and hafnocene polymers ranging from 4.1 to 10.

As typical, the polymers do show inhibition to normal human calls as evaluated by results for the WI-38 cells that are normal healthy human cells. The monomers are non-toxic to the highest level tested towards the normal cell line. The polymers are typically less toxic and compared with cisplatin much less toxic to the normal human cells.

In summary, all four polymers exhibit decent inhibition of several of the cell lines with the titanocene, hafnocene, and vanadocene polymers offering superior inhibition of the cell lines compared with the zirconocene polymer. We have no explanation for this.

4. Conclusions

High polymers were synthesized employing the interfacial polymerization of the disalt of 3,5-purinedicarboxylic acid with Group VA and vanadocene dichlorides employing commercially available reactants. The synthetic approach allows the scale-up from grams to tons. Chain length and yield appear to increase as the "softness" of the metal atom increases. Infrared spectroscopy is consistent with the products being polyesters with a non-bridging distorted tetrahedral geometry about the metal atom. MALDI MS gives ion fragments to two units with isotopic abundances consistent with the presence of one and two metal atoms in the ion fragment clusters.

The polymers and monomers were tested against a battery of cancer cell lines-two pancreatic cancer cell lines, two breast cancer cell lines, and one prostate and one colon cancer cell line, all of the polymers exhibit decent EC_{50} values and most CI_{50} values greater than two with the titanocene, hafnocene and vanadocene polymers showing superior CI_{50} values compared with the zirconocene polymer.

References

[1] C. Carraher. Organotin polymers, in Macromolecules Containing Metal and Metal-Like Elements, Vol. 4. Group IVA Polymer, *Wiley*, Hobokin, 2005.
[2] C. Carraher, G. Scherubel. Synthesis of poly[alkylene(arylene)oxystannanes] using a new modified system. *Makromolekular Chemie*, **1972**,*152*, 61-69.

[3] C. Carraher, G. Scherubel. Synthesis of polyoxystannyloxyalkylenes. *J. Poly. Sci.,* **1971**, *A-1, 9*, 983-990.
[4] C. Carraher, G. Scherubel. Synthesis of poly(tin ethers) employing alkoxides. *Makromolekular Chemie*, **1972**, *160*, 259-261.
[5] G. Barot, K. Shahi, M. Roner, C. Carraher. Synthesis, anomalous fiber formation, and preliminary anticancer study of the organotin polyether derived from 2-butyne-1,4-diol. *J. Polym. Mater.*, **2006**, *23*, 423-436.
[6] G. Barot, M. Roner, Y. Naoshima, K. Nagao, K. Shahi, C. Carraher. Synthesis, Structural Characterization, and Preliminary Biological Characterization of Organotin Polyethers Derived from Hydroquinone and Substituted Hydroquinones. J. Inorg. Organomet. Polym., 2009, 19, 12-27.
[7] M. Roner, K.R. Shahi, G. Barot, A. Battin, C. Carraher, Preliminary Results for the Inhibition of Pancreatic Cancer Cells by Organotin Polymers, *J. Inorg. Organomet. Polym.,* **2009**, *19*, 410-414.
[8] M. Roner, C. Carraher, K. Shahi, G. Barot. Antiviral Activity of Metal-Containing Polymers-Organotin and Cisplatin-Like Polymers. *Materials*, **2011**, *4*, 991-1012.
[9] G. Barot, K. Shahi, M. Roner, C. Carraher. Synthesis, structural characterization, and ability to inhibit cancer growth of a series of organotin poly(ethylene glycols). *Journal of Inorganic and Organometallic Polymer Materials*, **2007**, *17*, 595-603.
[10] C. Carraher, Y. Naoshima, K. Nagao, M. Roner, A. Zhao, G. Barot, A. Battin. Antibacterial, Yeast and Fungal Polymeric Materials Derived from Organotin-Containing Materials. *J. Polym. Mater*., **2011**, *28*, 303-336.
[11] C. Carraher, A. Battin, K. Shahi, M. Roner. Synthesis, structural characterization, and initial evaluation as anticancer drugs of dibutyltin polyamines derived from various 4,6-diaminopyrimidines. *J. Inorg. Organomet. Polym.,* **2007**, *17*, 631-639.
[12] C. Carraher, F. Blum, M. Nair, G. Barot, A. Battin, T. Fiore, C. Pellerito, M. Scopelliti, A. Zhao, M. Roner, L. Pellerito. Solid State Analysis of Metal-Containing Polymers Employing Mossbauer Spectroscopy, Solid State NMR and F EI TOF MALDI MS. *J. Inorg. Organomet. Polym.*, **2010**, *20*, 570-585.
[13] A. Zhao, K. Shahi, M. Roner, G. Barot, T. Fiore, C. Pellerito, M. Scopelliti, L. Pellerito, C. Carraher. Ciprofloxacin Polymers Derived from Diallyltin and Divinyltin Dihalides. *J. Polym. Mater.*, **2008**, 25, 213-236.
14. M. Roner, C. Carraher, J. Roehr, K. Bassett. Antiviral and anticancer activity of organotin polymers and reactants derived from norfloxacin and ampicillin. *J. Polym. Mater.,* **2006**, *23*, 153-159.
15. C. Carraher, L. Lanz. Synthesis and initial structural characterization of organotin polymers containing Norfloxacin (TM). *J. Polym. Mater*., **2003**, *20*, 91-99.
16. C. Carraher, K. Morie, Synthesis of organotin polyesters containing ticarcillin, J. Polym. Mater., 2004, 21, 383-391.
17. C. Carraher, T. Sabir, M. Roner, K. Shahi, R. Bleicher, J. Roehr, K. Bassett. Synthesis of organotin polyamine ethers containing acyclovir and their preliminary anticancer and antiviral activity. *J. Inorg. Organomet. Polym.,* **2006**, *16*, 249-257.
18. C. Carraher, D. Siegmann-Louda. Organotin macromolecules as anticancer drugs. Macromolecules Containing Metal and Metal-Like Elements, Vol 3. Biomedical Applications, *Wiley, Hobokin*, 2004.

19. C. Carraher, R. Schwarz, J. Schroeder, M. Schwarz. Organotitanium Polydyes Derived from Phenylsulfonphthalein Dyes, and Congo Red, Eriochrome Black-T, Nigrosine and Indigo Carmine-Synthesis and Doping Characteristics. *J. Macromol. Sci. Chem.,* **1981**, *A15*, 773-785.
20. C. Carraher, L.P. Torre, H.M. Molloy. Synthesis and Structural Characterization of Titanium Polyoximes. *J. Macromol. Sci. Chem.,* **1981**, *A15*, 757-771.
21. C. Carraher, M. Christensen, J.A. Schroeder. Physical Characterization of Titanium Poly-Ferrocene Oximes. *J. Macromol. Sci. Chem.,* **1977**, *A11*, 2021-2028.
22. C. Carraher, G. Burrish. Synthesis and Initial Thermal Characterization of Titanium Polyferrocene Ethers. *J. Macromol. Sci. Chem.,* **1976**, *A10*, 1457-1465.
23. C. Carraher, S. Bajah. Synthesis of titanium polyethers by the interfacial and aqueous solution techniques. *Polymer*, **1973**, *14*, 42-44.
24. C. Carraher, S. Bajah. Synthesis of Titanium Polyethers by Interfacial and Aqueous-Solution Techniques. *Polymer*, **1973**, *14*, 42-44.
25. C. Carraher, S. Bajah. Effects of base nature, base concentration, and method of synthesis of titanium polyether. *British Polymer Journal*, **1975**, *7*, 155-159.
26. C. Carraher, R. Frary. Synthesis of Zirconium Poly-O-Amidoximes. *J. Polym. Sci. Chem.,* **1974**, *12*, 799-805.
27. C. Carraher, L. Jambaya. Initial Synthesis and Thermal Characterization of Hafnium Polyethers. *Angew Makromol. Chem.,* **1976**, *52*, 111-116.
28. C. Carraher, A. Battin, M. Roner. Effect of Bulk Doping on the Electrical Conductivity of Selected Metallocene Polyamines. *J. Inorg. Organomet. Polym.,* **2013**, *23*, 61-73.
29. M. Roner, C. Carraher, Jr., K. Shahi, Y. Ashida, G. Barot. Ability of Group IVB metallocene polyethers containing dienestrol to arrest the growth of selected cancer cell lines. *BMC Cancer*, **2009**, *9*, 358.
30. C. Carraher, M. Roner, K. Shahi, Y. Ashida, G. Barot. Synthesis, structural characterization, and anti-cancer evaluation of group IVB-metallocene polyethers containing the synthetic estrogen diethylstilbestrol. *J. Polym. Mater.,* **2007**, *24*, 357-369.
31. C. Carraher. Condensation Metallocene Polymers. *J. Inorg. Organomet. Polym.,* **2005**, *15*, 121-145.
32. C. Carraher, W.J. Scott, J. Schroeder, D.J. Giron. Poly(Cis-Dihalodiamine Platinum(Ii)) Compounds - Synthesis and Biological-Activity. *J. Macromol. Sci. Chem.,* **1981**, *A15*, 625-631.
33. D. Siegmann-Louda, Carraher, C.. Effect of poly(dichloro-2-chloro-p-phenylenediamineplatinum(II) on normal and transformed cells. *J. Polym. Mater.,* **1987**, *4*, 29-34.
C. Carraher. Organoantimony-Containing Polymers. *J. Polym. Mater.,* **2008**, *25*, 35-50.
35. T. Sabir, C. Carraher. Synthesis of triphenylantimony and triphenylbismuth-containing polyether amines containing acyclovir. *J. Polym. Mater.,* **2006**, *4*, 403-413.
36. D. Siegmann-Louda, C. Carraher, Q. Quinones, G. McBride. Preliminary evaluation of organoarsenic and organoantimony polymers derived from cephalexin as potential anticancer drugs. *Polym. Mater. Sci. Eng.,* **2003**, *88*, 390-392.
37. C. Carraher, M. Nass, D. Giron, Antimony polyamines as antibacterial additives. *Org. Coat. Applied Polym. Sci. Proceedings*, **1983**, *48*, 523-527.
38. C. Carraher, L. Hedlund. Synthesis and characterization of antimony (V) polyoximes. *J. Macromol. Sci.-Chem.* **1980**, *A14*, 713-728.
39. C. Carraher, W. Venable, H. S. Blaxall, J. E. Sheats. Synthesis and characterization of antimony (V)-polycobalticinium exters. *J. Macromol. Sci.-Chem.,* **1980**, *A14*, 571-579.

40. C. Carraher, H. S. Blaxall. Synthesis and solution characterization of antimony polyesters. *Angew. Makromol. Chemie*, **1979**, *83*, 37-45.
41. C. Carraher, M. Naas, D. J. Giron, D. R. Cerutis. Structural and biological characterization of antimony V polyamines. *J. Macromol. Sci.-Chem.*, **1983**, *A19*, 1101-1120.
42. M. Roner, C. Carraher, A. Moric, N. Trang, N. Truong, Z. Islam, A. Morrison. Inhibition of pancreatic cancer cell lines by group IVB and group VA polymers derived from glycyrrhetinic acid, 3,5-pyridinedicarboxylic acid, and histamine. *Polym. Mater. Sci. Eng.*, **2012**, *107*, 325-328.
43. W. Lee, J. Elliott, R. Brownsey. Inhibition of acetyl-CoA carboxylase isoforms by pyridoxal phosphate. *J. Bio. Chem.*, **2005**, *280*, 41835-43.
44. K. McCann, J. Laane. Ramon and infrared spectra and theoretical calculations of dipicolinic acid, dinicotinic acid, and their dianions. *J. Mol. Struc.*, **2008,** *890*, 346-358.
45. N. Ogata. Synthesis of polyamides and polyesters having various functional groups. *J. Macromol. Sci. Chem.*, **1979,** *A13*, 477-501.
46. C. Marvel, H. Vogel. Polybenzimidazoles. **1965** US Pat. 3174947.
47. H. Hoff, A. Krieger. Polyamides from heterocyclic dicarboxylic acids. *Makromolecular Chemie*, **1961**, *47*, 93-113.
48. H. Shizunobu, N. Yasuhiko. Synthesis of polyesters containing pyridine rings in the main chains. *Kobunshi Kagaku*, **1967**, *24*, 215-223.
49. G. I. Braz, I. E. Kardash, V. S. Yakubovick, G. V. Myasnikova, A. Ardashnikov, A. Oleinik, A. Pravednikov, A. Yakubovick. Polybenzosazoles, their synthesis and thermal degradation. *Vysokomoleculyarnye Soedineniya*, **1966**, *8*, 272-277.
50. P. Hergenrother, W. Wrasidlo, H. Levine. Polybenzothiazoles. I. Synthesis and preliminary stability evaluation. *J. Polym. Sci, Part A.*, **1965,** *3*, 1665-1674.
51. A. Szorcsik, L. Nagy, M. Scopelliti, A. Deak, L. Pellerito, G. Galbacs, M. Hered. Preparation and structural characterization of $[Ph3Sn(IV)]^+$ complexes with pyridine-carboxylic acids or hydroxypyridine, -pyrimidine and –quinolone. *J. Organometallic Chem.*, **2006**, *691*, 1622-1630.
52. A. Szorcsik, L. Nagy, A. Deak, M. Scopelliti, Z. Fekete, A. Csaszar, C. Pellerito, L. Pellerito. Preparation and structural studies on the $Bu_2Sn(IV)$ complexes with aromatic mono- and dicarboxylic acids containing hetero {N} donor atmo. *J. Organometallic Chem.*, **2004,** *689*, 2762-2769.
53. C. Ma, J. Li, R. Zhang. Synthesis and crystal structures of polymeric ionic triorganotin esters of 3,5-pyridinedicarboxylic acid and 5-nitroisophthalic acid. *J. Coordination Chem.*, **2006,** *59*, 1891-1904.
54. H. Qisong, H. Benfeng, L. Qiuyan, L. Taiqi. Synthesis, crystal structure and fluorescence property of terbium coordination polymers with pyridine-3,5-dicarboxylic acid. *Materials Science Foru*, **2010**, *663*, 72-75.
55. Q. Shi, F. Liang. Synthesis, crystal structure and characterization of a novel terbium fluorescent coordination polymer. *Xiyou Jinshu Cailiao Yu Gongcheng*, **2010**, *39*, 1202-1205.
56. V. Chandrasekhar, R. Thirumoorthi. Self-assembly of triorganotin (V) moieties with 1,2,4,5,-benzenetetracarboxylic acid: synthesis, characterizations andinfluence of solvent on the molecular structure. *J Organometal Chem*, **2009**, *28*, 2096-2106.
57. J. Qiang, Z. Jiao, S. Xin. Synthesis and crystal structure of nickel 3,5-pyridine dicarboxylates. *Gaodeng Xuexiao Huaxue Xuebao*, **2010**, *31*, 1496-5101.
58. D. Lin, W. Kun-Miao, W. Guang-Ke, F. Rui-Bin, Z. Qu-Hua. Hydrothermal synthesis of two 3D lanthanide(III) organometallic polymers in the 3,5-dicarboxylicdicarboxylate system with

different coordination architecture. *Chinese J Structural Chem.*, **2010,** *29*, 618-1624.
59. D. Dongbin, G. Hui, B. Yan, Z. Guoqiang. Synthesis, crystal structure and luminescent properties of one coordination polymer of copper (II) achieved from pyridine-3-5 dicarboxylate. *J. Chem. Crystallography*, **2010**, *40*, 332-336.
60. Y. Song., J. Ji, G. Han, G. Zhang, Z. Han. Hydrothermal synthesis and structural characterization of a new coordination polymer [In(Pdc)(OH)(2,2'-Bipy)]In (H_2Pdc=3,5-pyridinedicarboxylic acid, 2,2'bipyridine). *Russian J. Coordination Chem.,* **2010,** *36*, 113-116.
61. J. Zhang, S. Chem, S. Xiang, J. Huang, L. Chen, C. Su. Heterometallic coordination polymer gels based on a rigid, bifunctional ligand. *Chem. Eur. J.,* **2011,** *17*, 2369-2372.
62. Z. Guo, Y. Li, W. Yuan, X. Zhu, X. Li, R. Cao. Synthesis, structures, and characterizations of two new indium (III) compounds from 1D, In-OH-In-OH chains and pyridinedicarboxylic ligands. *Eur. J. Inorg. Chem.,* **2008**, 1326-1331.
63. G. Liu, Y. Xu, X. Ren, S. Nishihara, R. Huang. Self-assembly of 3D 4d-4f coordination frameworks based on pyridine-3,5-dicarboxylic acid: synthesis, crystal structures and luminescence. *Inorganica Chimica Acta*, **2010**, *363*, 3727-32.
64. S. Monmoton, H. Lefebvre, F. Costa-Torro, A. Fradet. Hyperbranched poly[bis(alkylene)pyridinium]s. *Macromol. Chem. Phys.*, **2008,** *209*, 2382-2389.
65. E. Chekmeneva, C. Hunter, M. Packer, S. Turega. Evidence for partially bound states in cooperative molecular recognition interfaces. *J. Am. Chem. Soc.*, **2008,** *130*, 17718-17725.
66. C. Carraher. Polymer Chemistry, *Taylor and Francis*, **2014**, NY.
67. C. Carraher. Introduction to Polymer Chemistry, *CRC Press*, **2013**, Boca Raton, FL.
68. F. A. Cotton. Wilkinson, G. *Advanced Inorganic Chemistry*, 6th Ed., Interscience, **1999**, NY.
69. C. E. Carraher. Synthesis of zirconium polyesters. *Europ Polym J*, **1972**, *8*, 215-220.
70. A. Battin, C. E. Carraher. Effect of doping by exposure to iodine vapor on the electrical conductivity of the polyamine from titanocene dichloride and 2-nitro-p-phenylenediamine. *J Polym Mater*, **2008**, *23*, 23-33.
71. C. E. Carraher, A. Battin, M. R. Roner. Effect of electrical conductivity through the bulk doping of the product of titanocene dichloride and 2-nitro-1,4-phenhylenediamine. *J Funct Biomater*. **2011**, *2*, 18-30.
72. A. Gross, H. Alborzinia, S. Piantavigna, L. L. Martin, S. Wölfl and N. Metzler-Nolte. Vesicular disruption of lysosomal targeting organometallic polyarginine bioconjugates. Metallomics, **2015**, 7(2), 371-384.
73. A. Gross, H. Alborzinia, S. Piantavigna,L. L. Martin, S. Wölfl and N. Metzler-Nolte. Structure-activity relationship of trifluoromethyl-containing metallocenes: electrochemistry, lipophilicity, cytotoxicity, and ROS production. ChemMedChem. **2014**, 9(6), 1188-1194.
74. R. Havelek , P. Siman, J. Cmielova, A. Stoklasova, J. Vavrova, J. Vinklarek, J. Knizek and M. Rezacova. Differences in vanadocene dichloride and cisplatin effect on MOLT-4 leukemia and human peripheral blood mononuclear cells. Med Chem. **2012**, 8(4), 615-21.
75. S. Gómez-Ruiz S, D. Maksimović-Ivanić, S. Mijatović and G. N. Kaluđerović. On the discovery, biological effects, and use of Cisplatin and metallocenes in anticancer chemotherapy. Bioinorg Chem Appl., **2012**:140284. doi: 10.1155/2012/140284. Epub 2012 Jul 12.
76. D. Senthilnathan , S. Vaideeswaran, P. Venuvanalingam and G. Frenking. Antitumor activity of bent metallocenes: electronic structure analysis using DFT computations. J. Mol. Model., **2011**, Mar;17(3):465-475.
77. U. Olszewski, J. Claffey, M. Hogan, M. Tacke, R. Zeillinger, P. J. Bednarski, Hamilton G.

Anticancer activity and mode of action of titanocene C., Invest New Drugs, **2011**, Aug;29(4),607-614.
78. C. E. Carraher, A. Morrison, M. R. Roner, A. Moric, N. Trang. Synthesis and Characterization of Organotin Polyesters Derived from 3,5-Pyridinedicarboxylic Acid. *J. Inorg. Organomet. Polym.*, **2014**, *24*, 182-189.
79. Carraher, CE, Synthesis of titanium polyesters, J Polym Sci Pt A-1, 9, 3661-2670 (1971)
80. C. Carraher, T. S. Sabir, C. L. Carraher, Fundamentals of fragmentation matrix assisted laser desorption/ionization mass spectrometry, in Inorganic and Organometallic Macromolecules, Springer, NY, 2008, pgs. 329-350.
81. C. Carraher, T. Sabir, C. L. Carraher, Fragmentation matrix assisted laser desorption/ionization mass spectrometry-basics. *J. Polymer. Mater*, **2006**, *23*, 143-151.
82. C. Carraher, G. Barot, A. Battin, Reactions between the matrix and ion fragments created from the MALDI MS or organotin-containing polymers. *J. Polym. Mater*, **2009**, *26*, 17-31.
83. C. E. Carraher, M. R. Roner. Organotin polymers as anticancer and antiviral agents. *J. Organometal. Chem*, **2014**, *751*, 67-82.
84. M. R. Roner, K. Shahi, G. Barot, A. Battin, C. E. Carraher. Preliminary Results for the Inhibition of Pancreatic Cancer Cells by Organotin Polymers. *J Inorg Organomet Polym*, **2009**, *19*, 410-414.
85. C. E. Carraher, M. Ayoub, M R. Roner, A. Moric, N. Trang. Synthesis, structural characterization, and ability to inhibit the growth of pancreatic cancer by organotin polymers containing chelidonic acid. *JCAMS*, **2013**, *1*, 65-73.
86. C. E. Carraher, N. T. Truong, M. R. Roner. Synthesis of organoarsenic, organoantimony, and organobismuth poly(ether esters) from reaction with glycyrrhetinic acid and their preliminary activity against pancreatic cancer cell lines. *JCAMS*, **2013**, *1*, 134-150.
87. C. E. Carraher, G. Barot, S. W. Vetter, G. Nayak, M. R. Roner. Degradation of the organotin polyether derived from dibutyltin dichloride and hydroxyl-capped poly(ethylene glycol) in trypsin and evaluation of trypsin activity employing light scattering photometry and gel electrophosphoresis. *JCAMS*, **2013**, *1*,1-6.
88. C. E. Carraher, G. Barot, K. Shahi, M. R. Roner. Influence of DMSO on the inhibition of various cancer cells by water-soluble organotin polyethers. *JCAMS*, **2013**, *1*, 294-304.
89. D. Siegmann-Louda, C. E. Carraher, F. Pflueger, J. R. Ross. Organometallic condensation polymers as anticancer drugs, in Functional Condensation Polymers. *Plenum, NY*, **2002**, Chapter 14.
90. R. Doucette, D. Siegmann-Louda, C.E. Carraher, A. Cardoso. Inhibition of Balb 3T3 cell as a function of metal for kinetin containing polymers. *Polym Mater Sci Eng*, **2004**, *91*, 564-566.
91. R. M. Roat-Malone. Bioinorganic Chemistry, 2ed. *Wiley, NY*, **2007**, pp. 19-20.
92. J. B. Waern, H. H. Harris, B. Lai, Z. Cai, M. M. Harding, C. T. Dillon, CT. Intracellular mapping of the distribution of metals derived from the antitumor metallocenes. *J. Bio. Inorg. Chem.*, **2005**, *10(5)*, 443-452.
93. B. Ekwall, V. Silano, A. Paganuzzi-stammati, F. Zucco. Toxicity tests with mammalian cell cultures in short-term toxicity tests for non-genotoxic effects. *Wiley, NY,* **1990**.

CHAPTER 13

SYNTHESIS AND CHARACTERIZATION, INCLUDING CANCER CELL LINE INHIBITION, OF GROUP VA (GROUP 15)-CONTAINING POLYESTERS FROM REACTION WITH CAMPHORIC ACID[11]

Introductory Comments

For the proceeding article, data on the biological effects of the of the tested compounds was collected by me (Tables 9-12) and other members of the laboratory. The data was grouped as necessary to present the data in a meaningful manner, as described in the abstract for the article.

Authors: Carraher, Charles E., Michael R. Roner, Francesca Mosca, Alisa Moric-Johnson, **Lindsey C. Miller**, Jeffrey D. Einkauf, Floyd Russell, and Paul Slawek

Carraher, Charles E., Michael R. Roner, Francesca Mosca, Alisa Moric-Johnson, **Lindsey C. Miller**, Jeffrey D. Einkauf, Floyd Russell, and Paul Slawek (**2017**). "Synthesis and Characterization, Including Cancer Cell Line Inhibition, of Group VA (Group 15)-Containing Polyesters from Reaction with Camphoric Acid." Journal of Inorganic and Organometallic Polymers and Materials.

[11] Used with permission of Springer, 2017

Synthesis and Characterization, Including Cancer Cell line Inhibition, of Group VA-Containing Polyesters from Reaction with Camphoric Acid

Charles E. Carraher, Jr.,[a] Michael R. Roner,[b] Francesca Mosca,[a] Alisa Moric-Johnson,[b] Lindsey C. Miller[b] , Jeffrey D. Einkauf,[a] Floyd Russell[a] , Paul Slawek[a]

a. Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431 (carraher@fau.edu)

b. University of Texas Arlington, Department of Biology, Arlington, TX 76010; (roner@uta.edu)

Corresponding author: Charles E. Carraher, Jr., Florida Atlantic University, Department of Chemistry and Biochemistry, Boca Raton, FL 33431, 561-297-2107; 561-297-Fax (carraher@fau.edu)

Abstract Polyesters were rapidly synthesized employing interfacial polymerization from reaction of the salt of camphoric acid with Group VA triphenylmetallic dihalides. Yields range from 25 to 46 percent with chain lengths about 250. Infrared spectroscopy shows the formation of two new bands one assigned to the symmetrical M-O stretching and the second assigned to the asymmetrical M-O stretching. The bridging structure about the metal atom increases as the metal atom size increases. MALDI MS and proton NMR are consistent with the formation of the polyester structure. Ion fragment clusters to four to six units are identified. The polymers show good inhibition of a group of cancer cell lines including two pancreatic human cancer cell lines. In comparison with other metal/camphoric acid polymers, the metallocene polymers exhibit low EC_{50} to the nanogram/mL range, and CI_{50} values greater than one thousand for the hafnocene and zirconocene products. If this trend continues, the emphasis should be on the Group IVB metallocenes with respect to efforts to create anticancer drugs.

Keywords VA metal containing polymers; MALDI MS; pancreatic cancer; breast cancer; antimony-containing polymers; arsenic-containing polymers; bismuth-containing polymers

Dedication This paper recognizes the many contributions that Professor Pierre D. Harevy has made to the field of polymers and chemistry. He is a fellow comrade and it has been a pleasure to work with him on many projects.

1 Introduction

We have been engaged in the synthesis of a variety of metal-containing polymers for a variety of purposes. These efforts have been recently reviewed for organotin [1,2], platinum [3], Group VA metals [4], and Group IVB metallocene [5] containing polymers. One focus has been the synthesis and characterization of metal-containing polymers as anticancer agents, specifically inhibition of pancreatic cancer. This paper is part of an effort towards evaluation of the importance of the identity of the metal moiety towards inhibition of cancer cell lines.

The biological activity of organoarsenic, organoantimony and organobismuth compounds is well established [4.6-9]. The topic of arsenic, antimony and bismuth-containing polymers has been recently reviewed [4,7,8]. A number of different condensation polymers have been synthesized from simple condensation of Group VA organometallic dihalides or dinitrates with typical Lewis bases as diamines and diols [9-15].

Recently, an increased emphasis on synthesis of Group VA polymers occurred because of their ability to inhibit growth of human pancreatic cancer cell lines [16]. Much of this effort focused on coupling biologically active Lewis bases with organometallic reactants that also exhibit biological activity hoping for some synergetic effect between the two biologically active moieties.

Camphoric acid, CA, (Fig. 1) is obtained synthetically and from camphor so it is part of the green chemistry emphasis. It exists in three optically active forms-the most widely employed is the dextrorotatory or D form which is the form employed in the current study. It has been largely utilized because of its optical and medicinal properties. It is relatively non-toxic, usable in large dosages without serious negative biological consequences. It is believed to paralyze nerve-endings in the sweat glands and is employed in the treatment of "night-sweats" of tuberculosis patients. CA has few unwelcomed side effects. It is also used as a local antiseptic for the nose and bladder.

Fig. 1 D-Camphoric acid structure

There are numerous references to the inclusion of camphoric acid in polymers. Many of these are metal framework or coordination polymers [18-20]. There are several reports on the formation of polyesters and other related structures through polymerization with camphoric acid, camphoryl dichloride, and esters of camphoric acid. Zeu and coworkers reported the synthesis of liquid-crystalline polyesters from reaction of camphoroylbis(4-oxybenzoyl chloride) with aromatic and aliphatic diols or polymerization with camphoryl chloride with various diols [21]. Yourd described the synthesis of amorphous, cholesteric polyesters from reaction of camphoryl chloride with various diols [22]. Toy described the formation of stereoregular condensation polymers from reaction of camphoryl dichloride with various diols and with piperazine [23]. This was part of a larger study investigating the influence of structure and properties with respect to the effect of asymmetry polymer properties. Compared with the atactic materials, the optically active polymers had higher melting ranges and were more crystalline.

The current paper describes the synthesis and physical and initial anticancer characterization of Group VA polyesters produced from the reaction of the salt of camphoric acid with Group VA dihalides (Fig. 2).

Fig. 2 Repeat unit for the product of D-camphoric acid and triphenylbismuth dichloride where R_1 represents simple chain extension

2 Experimental

2.1 Synthesis

Reactions were carried out using the interfacial polycondensation technique, Briefly, an aqueous solution (10.0 ml) containing camphoric acid, (0.00200 mol) and sodium hydroxide (0.0040 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 room temperature, about 25 ^{o}C). Stirring was begun and a chloroform (for the triphenylantimony and triphenylarsenic and carbon tetrachloride for the triphenylbismuth) solution (10.0 ml) containing the Lewis acid dihalide (0.00200 mol) was rapidly added through a hole in the jar lid using a powder funnel. The resulting solution was blended for 5 seconds. The precipitate was recovered using vacuum filtration and washed several times with deionized water and chloroform (or carbon tetrachloride) removing unreacted materials and unwanted by-products. The solid was washed onto a glass Petri dish and allowed to dry at room temperature.

Triphenylarsenic dibromide (3313-89-1) was synthesized as described by Brickleband and co-workers [24]. Triphenylbismuch dichloride (594-30-9) and camphoric acid (560-09-8) were purchased from Aldrich Chemical Co., Milwaukee, WS; triphenylantimony dichloride (594-31-0) was purchased from Strem Chemical Co., Newburyport, MA. These were used as received.

2.2 Physical Characterization

Molecular weight was obtained using Light scattering photometry employing a Brice-Phoenix Universal Light Scattering Photometer Model 4000 with the products dissolved in DMSO. Infrared spectra were obtained employing attenuated total reflectance infrared spectroscopy utilizing a Thermo Scientific Nicolet iS5 FTIR equipped with an id5 ATR attachment. ^{1}H NMR spectra were obtained in d-6 DMSO employing Varian Inova 400 MHz amd 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 Da. A graphite matrix was employed. Graphite from a number 2 pencil was marked on the sample holder and sample placed onto the graphite mark.

2.3 Cell Testing

The toxicity of each test compound was evaluated as follows. Cells were seeded into a 96-well culture plate at a density of 20,000 cells per well in 100 µL of culture medium. Following a 24 h incubation period, the test compounds were added 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. Following incubation, Cell Titer-Blue reagent (Promega Corporation) was added (20ul/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 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 difference between the initial inhibition and final total inhibition is small with the region between initial inhibition to final total inhibition essentially linear.

3 Results and Discussion

3.1 Yields and Chain Lengths

Reaction occurs with all of the triphenyl VA dihalides. Table 1 contains the product yield, molecular weight and chain length (degree of polymerization, DP) for the products.

Table 1 Product yield, molecular weight and chain length as a function of Lewis Base

Reaction is rapid giving product within five seconds stirring. The rapid yield is a consequence of the relatively low activation energy for the reaction between the acid halides and Lewis bases [41-43]. Yield is decent. As in other polymerizations employing Group VA triphenylmetallic halides, additional product precipitates from the reaction system after the initial product is formed [4,11-16,25-28]. The initial precipitated amount is given in column 2 and the final total yield given in column 3 that include the values given in column 2. The arsenic product is a brown color 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 camphoric acid and triphenylantimony and triphenylbismuth moieties. Because the diacid form of CA is not a strong nucleophile, base is added converting CA to its salt which is a reasonably strong nucleophile allowing formation of the desired ester linkage.

3.2 Infrared Spectroscopy Results

Infrared spectral analysis was conducted on the products and reactants and assignments are based on prior work by us and others [25-28]. Results for the products appear in Tables 2 and 3. There is the presence of C-H stretching bands appearing in the aromatic and aliphatic regions about 3000 cm^{-1} (all vibrational bands are given in wave numbers, cm^{-1}) characteristic of both camphoric acid (below 3000) and the triphenylmetallic moieties (above 3000). New bands appear about 1260 (symmetrical stretch) and 740 (asymmetrical stretch) assigned to the formation of the M-O linkage. Other important bands and assignments appear in Tables 2 and 3.

Table 2 Infrared bands from the reactants and products

The esters can exist as bridging or distorted seven-bonded bridged structures (Fig. 3, left) and non-bridging or triangular bipyramidal structures (Figure 3, right) with respect to the geometry about the metal atom. Infrared spectroscopy is the easiest way to determine the structure about the metal [25-28]. Bridging asymmetric carbonyl absorptions are found around 1550-1590 (all infrared bands are given in cm^{-1}). Bridging symmetric carbonyl bands are found around 1410-1440. Non-bridging asymmetric carbonyl bands are found about 1600-1700; and the corresponding symmetric carbonyl band found about 1335-1345.

Fig. 3 Geometrical arrangements about the metal atom

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

Table 3 contains bands associated with bridging and non-bridging. Bands associated with non-bridging decrease as the metal atom increases in size while those associated with bridging increase as the metal atom increases. The larger atom size appears to allow more room for bridging to occur.

3.3 Proton NMR

NMR was conducted employing d-6 DMSO for the various products. Following describes results for CA, metallocene dichlorides, and the products with camphoric acid. Fig. 4 contains the structure indicating proton locations described for camphoric acid. Camphoric acid has six proton groupings. These are found at about 2.71 (proton 4), 2.48 (proton 2), 2.20 (protons 3), 1.14 (protons 8), 1.00 (protons 7), and 0.94 (protons 6). All of the Group VA triphenyl compounds have a band about 8. Triphenylarsenic dibromide has a band about 8.2. The polymer has a doubled about 7.8 from the triphenylarsenic moiety and bands at 2.72, 2.45, 2.20, 1.15, 1.05, and 0.98 from the camphoric acid moiety. Triphenylantimony dichloride has a band at 8.36 which is found in the polymer at 8.35. Bands form the camphoric acid moiety are found at 2.72, 2.46, 2.18, 1.15, 1.05 and 0.96. Triphenylbismuth dichloride has a band at 8.29. The polymer has a band at 8.30 from the triphenylbismuth moiety and bands at 2.66, 2.38, 2.20, 1.20, 1.10, and 0.95 from the camphoric acid moiety. The NMR bands in the polymer and monomers are similar consistent with polymer formation having minimal effect on the NMR. This is reasonable since the major protons are somewhat isolated from the formation of the O-M linkage. These results are consistent with other studies [25-28].

Fig 4 Structure of CA indicating positions of protons for assigned peaks

3.4 MALDI MS

While MALDI MS was developed for the analysis of non-volatile samples with special use in the identification of polymers its potential for the analysis of a wide range of soluble and insoluble materials has not been fulfilled [29-32]. This is largely due to the inability of many materials to be soluble in somewhat volatile liquids to a decent extent allowing close contact between the matrix material and analyzed sample. This has largely eliminated most synthetic high-polymers from being suitably analyzed employing MALDI MS.

For about a dozen years we and others have been employing MALDI MS for the identification of non-volatile metal and non-metal containing polymers. This has been recently reviewed [35,36]. This technique is not straight forward MALDI MS but focuses on the fragments created in the MALDI MS process. The technique should be applicable to any solid when the proper operating conditions are employed. There are some complications employing this technique to organometallics because of rearrangements and the particular sensitivity of organometallic moieties to laser radiation [35,36].

Graphite is employed as the matrix material because it gives good results with few interfering ion fragments produced above 500 Da which is the typical lower mass range employed in our studies [37,38]. Two general MALDI MS modes were employed. These are the reflective and linear mode. 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. Following are results for two of the polymers.

The major ion fragments for the triphenylantimony product with camphoric acid, CA, are given in Table 4 and shown in Figs. 5 and 6. Abbreviations are employed to describe tentative assignments. These are CA for camphoric acid minus two protons; Ph is the phenyl moiety; U is one repeat unit; 2U is two repeat units. Sodium is a common contaminant. All masses are given in Daltons, Da.

Fig 5 MALDI MS for the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 1300 Da in the reflective mode

Fig 6 MALDI MS for the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 1300 Da in the reflective mode

Table 4 Most abundant ion fragment clusters derived from the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 2400 Da

Similar to other metal-containing moieties contained within polymer backbones, attachments to the metal, here the phenyl moiety, can be lost at the sites of bond scission [35-38]. Ion fragments to four and a half repeat units are found consistent with the proposed structure. As in other studies, bond scission occurs at the heterogeneous backbone sites, namely the M-O, C-O, and M-O-C(O) sites. It is important to remember that metal bonds, including those connecting the current polyesters, are not stable to radiations employed in most MALDI spectrometers thus the main fragments are generally only a few units long, as in this study [33-38].

Antimony contains two isotopes allowing isotopic matches to be made. Table 5 contains such a match for three ion fragments containing a single antimony atom. The structures given in the top horizontal line correspond to the particular metal-containing ion fragment cluster. The agreement to the expected listed as "Standard", appearing in the two most left-hand columns, is reasonable consistent with the present of one antimony atom within these ion fragment clusters. Table 6 contains similar matches for ion fragment clusters containing two antimony atoms. Again, the results are reasonable consistent with these ion fragments containing two antimony atoms.

Table 5 Isotopic abundance matches for ion fragment clusters containing one antimony atom derived from the product of triphenylantimony dichloride and camphoric acid

Table 6 Isotopic abundance matches for ion fragment clusters containing two antimony atoms derived from the product of triphenylantimony dichloride and camphoric acid

The major ion fragments for the product of triphenylarsenic and camphoric acid are given in Table 7. Similar to the antimony ion fragment assignments, abbreviations are employed to describe tentative assignments for the arsenic products. These are CA for camphoric acid minus two protons; Ph is the phenyl moiety; U is one repeat unit; 2U is two repeat units. Again, sodium is a common contaminant and masses are given in Daltons, Da.

Table 7 Most abundant ion fragment clusters derived from the product of triphenylarsenic dibromide and camphoric acid over the mass range of 500 to 2400 Da

Ion fragments to five units are found. There is some loss of the phenyl unit on the metal. As in other studies, this loss occurs at the site of bond scission. The CA ring unit remains intact consistent with the mildness of MALDI MS.

As in other studies, bond scission preferably occurs at the hetero-bond sites in the polymer chain [25-28]. Fig. 7 shows these sites for the repeat unit from triphenylantimony/CA.

Fig. 7 Sites of preferred bond scission for the antimony polymer

3.5 Tumor Analysis

The battery of test cell lines used is given in Table 8. They represent a reasonably broad range of human cancers.

Table 8 Cell lines employed in the current study

Similar polymer drugs are cytotoxic and cell death is by necrosis [2,3,39,40]. Also, anticancer activity is brought about by the intact polymer and not through polymer degradation [3]. This is consistent with studies that show the polymers are stable in DMSO with half-chain lives, the time for the chain length to halve, generally in excess of 30 weeks [2,3,39,40]. Most organometallic compounds associate with polar solvents such as DMSO and the biological results may be influenced by the presence of the DMSO {2,3,33,39,40,44,46-48]. For similar organometallic polymers, the influence of DMSO on the tumor results is found to be minimal [2,4,44].

While different measures have been employed in the evaluation of cell line results the two most widely are employed in this study. The first measure is the concentration dose needed to reduce growth of a particular cell line. Several names are associated with this concentration. Here the term effective concentration, EC, will be used. 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}.

The EC_{50} values for the monomers and polymers are given in Table 9. For comparison, values for cisplatin are also given. Cisplatin is among the most widely employed anticancer drugs. It is highly toxic and as shown in Table 9 is toxic to the standard cell lines at very low concentrations.

Much of our recent effort has been on discovering compounds that inhibit pancreatic cancer because pancreatic cancer does not have a generally accepted "cure" [3,49-53]. Thus, two widely employed human pancreatic cancer cell lines are included in the current study. These are the AsPC-1 cell line which is an adenocarcinoma pancreatic cell line and represents about 80% of human pancreatic cancers. The PANC-1 cancer cell line which is an epithelioid carcinoma pancreatic cell line represents about 10% of human pancreatic cancers. All three of the triphenylmetallic/CA polymers show good inhibition of both pancreatic cancer cell lines. The inhibition of the pancreatic cancer cell lines is similar for both the ASPC-1 and PANC-1 cells indicating that inhibition by the polymers may be general for the other pancreatic cancers.

The breast cancer cell lines is 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 polymers

there was a marked difference between the ability to inhibit the two breast cancer cell lines dependent on polymer structure [2,3,54-57]. In the current study there is little difference in the ability to inhibit the two cell lines by the polymers. All three of the polymers exhibit good inhibition of the prostrate cancer cell line and colon cancer cell line [58,59].

In summary, based on EC_{50} values, the polymers show good ability to inhibit a variety of cancer cell lines.

Table 9 EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values given in ( ) are Standard Deviations for Each Set of Measurements

Based on EC_{50} values, all polymers exhibit good inhibition of the tested cell lines. The Lewis base, CA, and organometallic monomers inhibit all of the cell lines but to a lesser degree compared with the polymers. Thus, the combination of the Group VA moieties with CA produce polymers that exhibit better inhibition of the cell lines compared with monomers alone.

Another measure of the potential use of compounds as anticancer compounds 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 terms are employed to describe similar calculations. Here, the term chemotherapeutic index, CI, is used. The CI_{50} is the ratio of the EC_{50} for the NIH 3T3 or WI-38 standard cell lines divided by the EC_{50} for the particular test cell. Results are given in Table 10.

The current study of CI_{50} values has two parts, one evaluation of the CI as a measure of ability to inhibit cell growth and the second use of the standard cell lines. Two cell lines are typically employed in the evaluation of the effectiveness of compounds to arrest the growth of tumor cell lines. These two cell lines are used in the current study and are the NIH 3T3 and WI-38 cell lines. NIH 3T3 cells are mouse embryo fibroblast cells. They are part of a group of cell lines that are referred to as partially transformed cells in that they are immortal unlike normal cells. 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. They have 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. Even so, when there is a difference between the results, greater confidence is given to the WI-38 cell results. In the present study the CI_{50} values for the WI-38 and NIH 3T3 cell lines are similar consistent with the use of either cell lines as the standard being permissible.

The CI_{50} values derived from values given in Table 10 are derived from values given in Table 9. The focus here is only on the CI_{50} values for the polymers. It is preferential to find large values meaning that the tendency to inhibit is preferential compared to the standard cell lines. In general, CI values of two and greater are considered significant. There are no values greater than two.

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

Some scientists favor EC_{50} values while others favor the CI_{50} values [2,3]. For the current study, the polymers exhibit good inhibition of the cancer cell lines, including the pancreatic cancer cell lines, based on EC_{50}, values but only small values based on the CI_{50} values.

3.6 Comparison

The current study is part of a larger investigation that evaluates the identity of the metal atom with respect to their ability to inhibit cancer cell growth. Recently we synthesized the analogous products from camphoric acid but with different metal-containing acid halides [60,61]. Following is a brief comparison of these results. Values for the current study are added for ready reference.

Table 11 contains the EC_{50} values for the various metal-containing polymers. There is a clear difference as the metal is changed with the cancer cell values for the metallocene polymers clearly superior followed by the organotin values followed by the values for the present study. The fact that the metallocene values are the lowest is not totally unexpected since the metallocenes themselves are known to offer good anticancer activity. The anticancer activity of metallocene-containing products is well known since the initial report by Kopf-Maier and Kopf in 1979 of the activity of titanocene dichloride to control cancer cell growth [62]. The anticancer activity of such metallocene-containing products has been recently reviewed [63-68]. Titanocene dichloride underwent Phase I clinical trials. The trials indicated a dose-limiting side effect associated with nephrotoxicity and a number of unwanted physical side effects including nausea, reversible metallic taste, pain during infusion, hypoglycemia, with these features undesirable. Counter, the absence of an effect on proliferative activity of the bone marrow, generally a dose-limiting side effect, was positive. Some phase II clinical trials were undertaken with patients with breast metastatic carcinoma [69] and advanced renal cell carcinoma [70]. Unfortunately low activity discouraged further clinical study. It is interesting that the order of activity is Hf>Zr>Ti for the current polymers so that future studies might emphasize the hafnocene and zirconocene products rather than titanocene-derived materials. The EC_{50} values for the metallocene products approach the nanogram/mL concentration level.

Table 11 EC_{50} values as the metal-containing moiety is varied

There is also a trend with respect to the toxicity of the standards 3T3 and WI-38 cells such that the polymer toxicity towards the standards is least for the metallocenes followed by the organotin and the greatest toxicity by the Group VA polymers.

The corresponding CI_{50} values are given in Table 12. Only the metallocene products give CI_{50} values greater than two and in their case several are 1000 and greater. These are among the largest values observed. The values for the organotin and Group VA polymers are all less than two.

Table 12 CI_{50} values calculated from data given in Table 11

Based on EC_{50} values all of the polymers exhibit good inhibition of all of the cancer cell lines with the values being Group VA>organotin>metallocenes with the EC_{50} in the nanogram/mL range for the metallocenes. Based on the CI_{50} values the metallocene polymers are clearly the best. For this set of polymers, it is clear that metallocene polymers give the lowest EC_{50} and highest CI_{50} and are good candidates for live animal studies. Such studies are planned.

Two additional notes are appropriate. First, with the exception of triphenylarsenic dibromide, all of the reactants are commercially available and the interfacial polycondensation process is industrially employed in the production of aramid fibers and polycarbonates so that ready production of the materials is possible [41-43]. Second, one major problem with the metallocene materials is their lack of solubility [63-70]. We have recently reported the synthesis of water-soluble Group IVB metallocene polymers employing reactions between the metallocene dichlorides and poly(ethylene glycols) [71,72]. Further, we have synthesized a number of metallocene polymers that are soluble in DMSO [2,3]. These efforts should afford researchers alternatives to producing soluble metallocene materials for biological testing.

3.7 Summary

Synthesis of polymers is rapid (about 5 seconds) employing largely commercially available reactants utilizing the interfacial polymerization of the salt of camphoric acid and Group V triphenylmetallic dihalides. The use of commercially available reactants and the interfacial polymerization that is employed industrially to make polycarbonates and aramids allow the scale-up from grams to tons. Yields range from 25 to 46 percent with chain lengths about 250. Infrared spectroscopy shows the formation of two new bands one assigned to the symmetrical M-O stretching and the second assigned to the asymmetrical M-O stretching. The bridging structure about the metal atom increases as the metal atom increases with the increased size presumably allowing greater room for bridging to occur. MALDI MS and proton NMR are consistent with the formation of the polyester structure. Ion fragment clusters to five and six units are identified and for the antimony polymer, isotopic abundance is consistent with the presence of antimony atoms within the ion fragment clusters. Tumor analysis based on EC_{50} is consistent with the polymers exhibiting decent inhibition of cancer cell growth including for the two pancreatic cancer cell lines. In comparison with other metal/camphoric acid polymers, the metallocene polymers exhibit low EC_{50} to the nanogram/mL range, and CI_{50} values greater than one thousand for the hafnocene and zirconocene products. If this trend continues, the emphasis should be on the Group IVB metallocenes with respect to efforts to create anticancer drugs.

References

1. C. Carraher, *Macromolecules Containing Metal and Metal-Like Elements, Vol. 4. Group IVA Polymer* (Wiley, Hobokin, 2005)
2. C. Carraher, M. R. Roner, J. Organomet. Chem. **751**, 67 (2014)

3. M. Roner, C. Carraher, K. Shahi, G. Barot, Materials **4**, 991 (2011)
4. C. Carraher, J. Polym. Mater. **25**, 35 (2008)
5. C. Carraher, J. Inorg. Organometal. Poly. **15**, 121 (2005)
6. K. Naka, Y. Chujo, Kanaku to Kogyo **60**, 520 (2007)
7. N. Karak, S. Maiti, S. Das, S. H. Dey, J. Polym. Mater. **20**, 237 (2003)
8. N. Karak, S. Maiti, J. Polym. Mater. **13**, 179 (1996)
9. N. Karak, S. Maiti, Angew. Makromol. Chemie. **265**, 5 (1999)
10. C. Carraher, *Bioactive Polymeric Systems* (Plenum, NY, 1985) p 651
11. T. Sabir, C. Carraher, J. Polym. Mater. **4**, 403 (2006)
12. C. Carraher, L. Hedlund, J. Macromol. Sci.-Chem. **A14**, 713 (1980)
13. C. Carraher, W. Venable, H. S. Blaxall, J. E. Sheats, J. Macromol. Sci.-Chem. **A14**, 571 (1980)
14. C. Carraher, H. S. Blaxall, Angew. Makromol. Chemie. **83**, 37 (1979)
15. C. Carraher, M. Naas, D. J. Giron, D. R. Cerutis, J. Macromol. Sci.-Chem. **A19**, 1101 (1983)
16. C. Carraher, N. Truong, M. Roner, A. Moric, N. Trang, J. Chin. Adv. Mater. Soc. **1**, 134 (2013)
17. C. Carraher, A. Campbell, M. Roner, Poly. Mater. Sci. Eng. **111**, 278 (2014)
18. L. Guang-Xiang, M. L. Wang, L. J. Su, Y. Zou, Y. Q. Wu, Wuji Huaxue Xuebao **27,** 1185 (2011)
19. D. N. Dybtsev, M. P. Yutkin, V. P. Fedin, Russ. Chem. Bull. **58**, 2246 (2009)
20. X. Q. Liang, D. P. Li, C. H. Li, X. H. Zhou, J. L. Zuo, Z. Z. You, Crys. Growth Design **10,** 2596 (2010)
21. V. V. Zuev, I. G. Denisov, S. S. Skorokhodov, Vysokomol. Soed. Serya A. **30,** 1534 (1988)
22. R. A. Yourd, Polym. Mater. Sci. Eng. **66**, 525 (1992)
23. M. S. Toy, J. Polym. Sci. Pt. A-1. **5,** 2481 (1967)
24. N. Brickleband, S. Godfrey, H. Lane, C. Mcauliffe, R. Pritchard, J-M. Moreno, J. Chem. Soc., Dalton Trans: Inorg. Chem. **23**, 3873 (1995)
25. C. Carraher, M. Roner, J. Dorestant. A. Moric-Johnson, M. H. Al-Huniti, J. Inorg. Organometal. Poly. Mat. **25**, 400 (2015)
26. C. Carraher, M. Roner, M. Ayoub, N. Pham, A. Moric, Inter. J Polym. Mat., **64**, 311 (2015)
27. C. Carraher, M. Roner, R. Thibodeau, A. Johnson, Synthesis, Inorg. Chim. Acta, **423**,123 (2014)
28. C. Carraher, M. Roner, N. Pham, A. Moric, J. Macromol. Sci, Part A. **51**, 547 (2014)
29. M. Barber, R. S. Bordoli, R. D. Sedwick, A. N. Tyler, Nature **293**, 270 (1981)
30. L. K. Liu, K. L. Busch, R. G. Cooks, Analyt. Chem. **53**, 109 (1981)
31. K. Tanaka, H. Waki, Y. Ido, S, Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass. Spectrom. **2**, 151 (1988)
32. M. Karas, F. Hillenkamp, Anal. Chem. **60,** 2299 (1988)
33. C. Carraher, F. Blum, M. Nair, G. Barot, A. Battin, T. Fiore, C. Pellerito, M. Scopelliti, A. Zhao, M. Roner, L. Pellerito, J. Inorg. Organomet. Polym. 570 (2010)

34. C. Carraher, T. S. Sabir, C. L. Carraher, *Inorganic and Organometallic Macromolecules* (Springer, NY, 2008), p. 329
35. C. Carraher, T. Sabir, C. L. Carraher, J. Polym. Mater. **23**, 143 (2006)
36. C. Carraher, G. Barot, A. Battin, J. Polym. Mater. **26**, 17 (2009)
37. C. Carraher, M. Roner, C. L. Carraher, R. Crichton, K. Black, J. Macromol. Sci. Part A. **52**, 867 (2015).
38. C. Carraher, V. Suresh, M. Roner, J. Polym. Mater. **32,** 151 (2015)
39. C. Carraher, *Macromolecules Containing Metal and Metal-Like Elements*, Vol. 4. Group IVA Polymer, (Wiley, Hobokin, 2005), p. 263
40. C. Carraher, D. Siegmann-Louda, *Macromolecules Containing Metal and Metal-Like Elements, Vol 3. Biomedical Applications* (Wiley, Hobokin, 2004), p 57
41. F. Millich, C. Carraher, *Interfacial Synthesis* (Dekker, New York, 1977)
42. F. Millich C. Carraher, *Interfacial Synthesis. Vol. II.* (Dekker, New York, 1977)
43. C. Carraher, J. Preston, *Interfacial synthesis* (Dekker, New York, 1977)
44. C. Carraher, G. Barot, K. Shahi, M. Roner, J. Chim. Adv. Mater. Soc. **1**, 294, 2013
45. C. Carraher, *Polymer Chemistry* (Taylor and Francis, NY, 2014)
46. H. Ohtaki, Pure Appl. Chem. **59,** 1143, 1987
47. K. Gjevig Jenson, A. Onfelt, M. Wallin, V., Lidumas, O. Andersen, Mutagenessis **6**, 409, 1991
48. R. Corriu, G. Dabosi, M. Martineau, J. Organomet. Chem. **186**, 25, 1980
49. C. Carraher, M. Roner, K. Shahi, G. Barot, Materials **4**, 801,2011
50. C. Carraher, M. Roner, K. Shahi, G. Barot, Materials **4**, 804, 2011
51. C. Carraher, M. Roner, Materials **2,** 1558, 2009
52. C. Carraher, M. Roner, K. Shahi, G. Barot, A. Battin, J. Inorg. Ogranomet. Polym. **19**, 410, 2009
53. C. Carraher, M. Roner, A. Moric-Johnson, L. Miller, G. Barot, N. Sookdeo, J. Macromol. Sci. Part A. **53**, 63, 2015
54. C. Carraher, M. Roner, M. Ayhoub, N. Pham, A. Moric-Johnson, Polym. Mater. Polym. Biomat. **64**, 311, 2015
55. C. Carraher, M. Roner, K, Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin, N. Trang,. N. Sookdeo, Z. Islam, Internat. J. Poly. Mater. **64**, 800, 2015
56. G. Barot, M. Roner, Y. Naoshima, K, Nago, K. Shahi, C. Carraher, J. Inorg. Organometal. Polym. **19**, 12, 2009
57. C. Carraher, M. Roner, K. Shahi, Y. Ashida, G. Barot, J. Inorg. Organometal. Polym. **18**, 180, 2008
58. C. Carraher, M. Roner, K. Shahi, A. Moric-Johnson, L. Miller, G. Barot, A. Battin, N. Trang, M. Alhuniti, J. Inorg. Organometal. Polym. **25**, 386, 2015
59. C. Carraher, M. Roner, L. Miller, K. Shahi, N. Trang, A. Moric-Johnson, G. Barot, A. Battin, M.Alhuniti, JCAMS. **2**, 303, 2014
60. C. Carraher, M. R. Roner, unpublished results.
61. C. Carraher, M. Roner, A. Moric-Johnson, L. Miller, A. G. Campbell, Polym. Mater. Sci. Eng. **112** ,347, 2015
62. H. Kopf, P. Kopf-Maier, Angew. Chem., Int. Ed. Engl. **18**, 477, 1979

63. E. Melendez, Inorg. Chim. Acta. **393**, 36, 2012
64. G. Gasser, I. Ott, N. Metzler-Nolte, J. Med. Chem. **54**, 3, 2011
65. S. Gomez-Ruiz, D. Maksimovic-Ivanic, S. Mijatovic, G. Kaluderovic, Bioinorganic Chem., Applications ID#140284, 2012
66. P. M. Abeysinghe, M. M., Harding, Dalton Trans. 3474, 2007
67. I. Kostova, Anti-Cancer Agents Med. Chem. **9**, 827, 2009
68. U. Olszewski, G. Hamilton, Anti-Cancer Agents in Med. Chem. **10**, 302, 2010
69. N. Kroger, U. R. Kleeberg, K. Mross, L. Edler, G. Sab, K. Hossfeld, Onkologie **23**, 60, 2000
70. G. Lummen, H. Sperling, H. Luboldt, T. Otto, H. Rubbe, Cancer Chemotherapy Pharmac. **42(5),** 415, 1998
71. C. Carraher, M. R. Roner, L. Reckleben, K. Black, J. Frank, R. Crichton, F. Russell, A. Moric-Johnson, L. Miller, J. Macromol. Sci. Part A. **53**, 394, 2016
72. C. Carraher, M. R. Roner, K. Black, J. Frank, A. Moric-Johnson, L. Miller, F. Russell, J. Chin. Adv. Mater. Soc., in press.

Tables

Table 1 Product yield, molecular weight and chain length as a function of Lewis Base

Lewis Base	% Initial Yield	Total Yield	Mol. Wt.	DP
Ph_3AsBr_2	19	25	1.4×10^5	250
Ph_3SbCl_2	11	46	1.5×10^5	300
Ph_3BiCl_2	6	38	1.5×10^5	230

Table 2 Infrared bands from the reactants and products

Band Assignment	Camphoric Acid	Ph_3AsBr_2	Ph_3As/ Polymer	Ph_3SbCl_2	Ph_3Sb/ Polymer	Ph_3Bi/ Polymer
CH ip st		3064	3055	3060	3060	3056
CH Sym St Aliph	2960, 2942		2967,2940		2950,2940	2970,2934
CH Asym St Aliph	2886, 2824		2890,2882		2862,2820	2880
C=O Asy St	1688		1637		1636	
CH_2 Sym St	1475		1484		1475	1472
C=C St		1465	1459	1460	1469	1460
M-Ph		1438	1439	1440	1436	1437
C=C ip Wag		1362	1360		1361	1374
M-O Asy St			1236		1279	1274
C-C		1178,1152	1182,1161	1152	1152	1170

M-Ph Sym St	1050	1086	1048	1060	1055
Ring Vib	997	998	995	995	986
M-O Sym St		739		730	728
Aryl op	690	689	690	686	689

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

Ph_3M Moiety	**Asym bridging**	**Sym bridging**	**Asym Non-bridging**	**Sym Non-bridging**
Ph_3As	1583 (s)	--	1637 (l)	1337 (m)
Ph_3Sb	1572 (m)	1450 (m)	1636 (m)	1342 (m)
Ph_3Bi	1559 (l)	1450 (m)	--	1340 (m)

Where l = large, m= moderate, s= small; -- = not observed

Table 4 Most abundant ion fragment clusters derived from the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 2400 Da

Mass,Da/ Linear	Mass,Da/ Reflective	(Tentative) Assignment
553	551	U
571	571	U+Na
588		U+O,Na
	616	U+CO_2,Na
716	716	U+CA-2O
	727	U+CA,Na-CO_2
735		U+CA-O
781	782	U+PhSb,O
	840	U+ Ph_3Sb,O
857		U+Ph_2Sb,2O
	873	U+ Ph_2Sb,CO_2
	986	U+ Ph_3Sb,CO_2,O,Na
1032	1031	U+ Ph_2Sb,O,Na
	1050	U+ Ph_2Sb,Na
1060		2U-CO_2
	1127	2U+Na
	1157	2U+CO_2,Na
	1207	2U+CA-2CO_2
1250	1254	2U+CA-CO_2
	1333	2U+ Ph_2Sb,CO_2
1449		2U+ Ph_3Sb
	1471	2U+ Ph_3Sb,Na
1525	1531	2U+ Ph_3Sb,CO_2,O,Na
	1557	2U+ Ph_3Sb,2CO_2
1607		3U-CO_2
1647		3U
	1678	3U+Na
1723	1724	3U+CO_2,Na
1807		3U+CA-CO_2
	1814	3U+CA-2O
	1829	3U+CA,Na-CO_2
1991		3U+ Ph_2Sb,CO_2,Na
2035		3U+ Ph_3Sb,O,Na
	2096	3U+ Ph_3Sb,2CO_2
2119	2114	3U+ Ph_3Sb,2CO_2,Na
2136		4U-Ph

2242	$4U+CO_2$
2339	$4U+CA-CO_2,O$

Table 5 Isotopic abundance matches for ion fragment clusters containing one antimony atom derived from the product of triphenylantimony dichloride and camphoric acid

Standard		**$U+CO_2,Na$**		**U+CA-2O**		**$U+CA,Na-CO_2$**	
Da	**% Rel Abu**	**Da**	**% Rel Abu**	**Da**	**% Rel Abu**	**Da**	**% Rel Abu**
121	100	616	100	716	100	727	100
123	75	618	70	718	75	729	72

Table 6 Isotopic abundance matches for ion fragment clusters containing two antimony atoms derived from the product of triphenylantimony dichloride and camphoric acid

Standard		**$U+CO_2,Na$**		**U+CA-2O**		**$U+CA,Na-CO_2$**	
Da	**% Rel Abu**	**Da**	**% Rel Abu**	**Da**	**% Rel Abu**	**Da**	**% Rel Abu**
242	67	780	66	984	68	1048	67
244	100	782	100	986	100	1050	100
246	37	784	42	988	40	1052	35

Table 7 Most abundant ion fragment clusters derived from the product of triphenylarsenic dibromide and camphoric acid over the mass range of 500 to 2400 Da

Mass,Da/ Linear	**Mass,Da/ Reflective**	**(Tentative) Assignment**
553	551	$U+CO_2,Na$
645	647	$U+CA-CO_2$
715	715	U+CA,Na
781	782	$U+Ph_3As$
	840	$U+ Ph_3As,CO_2,Na$
866	869	$U+ Ph_3As,2CO_2$

	980	2U
1031	1031	2U+CO_2
	1127	2U+CA-CO_2
	1158	2U+CA-O
1176		2U+CA
	1302	2U+ Ph_3As,2O
	1333	2U+ Ph_3As,CO_2,Na
1449		3U-O
	1471	3U
	1530	3U+CO_2,Na
1606		3U+CA,Na-2CO_2
1723	1721	2U+ Ph_2As,CO_2
1807		3U+ Ph_3As,CO_2
1884		4U-Ph
	1986	4U+Na
2034		4U+CO_2,Na
	2096	4U+CA-CO_2,O
	2114	4U+CA-CO_2
2136		4U+CA-O
	2155	4U+CA
	2242	4U+ Ph_3As
	2339	4U+ Ph_3As,2CO_2
	2387	5U+O-Ph
	2394	5U-CO_2

Table 8 Cell lines employed in the current study

Strain #	NCI Desig.	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	Embyro-continuous cell line of highly contact-inhibited cells	Fibroblast

AsPC-1	Human	Pancreatic cells	Adenocarcinoma
PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

Table 9 EC_{50} Concentrations (micrograms/mL) for the tested compounds. Values given in ( ) are Standard Deviations for Each Set of Measurements

Sample	*3T3*	*WI-38*	*PANC-1*	*AsPC-1*
Ph_3AsBr_2	>32000	>32000	>32000	390(11)
Ph_3As/CA	0.39(.1)	0.39(.1)	0.41(.1)	0.34(.1)
Ph_3SbCl_2	3600(21)	3500(34)	6500(18)	>32000
Ph_3Sb/CA	0.44(.8)	0.46(.07)	0.48(.08)	0.50(.1)
Ph_3BiCl_2	600(16)	790(90)	2100(10)	3700(22)
Ph_3Bi/CA	0.41(.1)	0.44(.1)	0.38(.1)	0.41(.1)
CA	1.1(.1)	1.1(.1)	1.1(.1)	1.2(.1)
Cisplatin	0.015(.01)	0.019(.01)	0.0023(.005)	0.0035(.005)

Sample	*3465/PC-3*	*7233/MDA*	*1507/HT-29*	*7259/MCF-7*
Ph_3AsBr_2	20.0(1.7)	21.4(1.0)	16.5(1.2)	24.6(2.1)
Ph_3As/CA	0.32((.1)	0.36(.1)	0.30(.1)	0.31(.1)
Ph_3SbCl_2	38(4)	12.4(1.1)	33.(3.1)	135(11)
Ph_3Sb/CA	0.51(.06))	0.46 (0.08)	0.49(.08)	0.53(.1)
Ph_3BiCl_2	2.4(.16)	1.4(.2)	2.2(.16)	1.6(.21)
Ph_3Bi/CA	0.44(0.1)	0.35(.1)	0.32(.1)	0.44(.1)
CA	1.1(.1)	1.2(.1)	1.3(.2)	1.2(.1)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

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

Sample	EC_{50} *WI-38*/ EC_{50}*AsPC*-1	EC_{50} *3T3*/ EC_{50}*AsPC*-1	EC_{50} *WI-38*/ EC_{50}*PANC*-1	EC_{50} *3T3*/ EC_{50}*PANC*-1
Ph_3AsBr_2	>82	>82	1	1
Ph_3As/CA	1.2	1.1	0.95	0.95
Ph_3SbCl_2	<0.11	<0.11	0.54	0.55
Ph_3Sb/CA	0.92	0.88	0.96	0.92
Ph_3BiCl_2	0.21	0.16	0.38	0.29
Ph_3Bi/CA	1.1	1.0	1.2	1.1
Cisplatin	1.3	4.2	8.3	6.5

Sample	EC_{50} *WI-38*/ EC_{50}*7233*	EC_{50} *3T3*/ EC_{50}*7233*	EC_{50} *WI-38*/ EC_{50}*7259*	EC_{50} *3T3*/ EC_{50}*7259*
Ph_3AsBr_2	>1495	>1495	>82	>82
Ph_3As/CA	1.1	1.1	1.3	1.3
Ph_3SbCl_2	282	26.7	<0.11	<0.11
Ph_3Sb/CA	1.0	0.96	0.87	0.83
Ph_3BiCl_2	564	375	494	375
Ph_3Bi/CA	1.3	1.2	1.2	0.93
Cisplatin	6.6	5.2	3.3	2.6

Sample	EC_{50} *WI-38*/ EC_{50}*1507*	EC_{50} *3T3*/ EC_{50}*1507*	EC_{50} *WI-38*/ EC_{50}*3465*	EC_{50} *3T3*/ EC_{50}*3465*
Ph_3AsBr_2	>1939	>1939	>1600	>1600
Ph_3As/CA	1.3	1.3	1.2	1.2
Ph_3SbCl_2	0.54	0.55	92	94.7
Ph_3Sb/CA	0.93	0.90	0.90	0.86

Ph_3BiCl_2	359	273	329	250
Ph_3Bi/CA	1.4	1.3	1.1	0.93
Cisplatin	4.6	3.7	4.3	3.4

Table 11 EC_{50} values as the metal-containing moiety is varied

Polymer	**3T3**	**WI-38**	**PAN-1**	**AsPC-1**
Cp_2Ti/CA	3.0	2.5	0.085	0.056
Cp_2Zr/CA	3.0	2.5	0.0024	0.0020
Cp_2Hf/CA	2.9	2.2	0.0019	0.0015
Me_2Sn/CA	0.084	0.054	0.061	0.059
Et_2Sn/CA	0.047	0.054	0.043	0.042
Bu_2Sn/CA	0.045	0.055	0.049	0.084
Oc_2Sn/CA	0.061	0.043	0.048	0.051
Ph_2Sn/CA	0.047	0.054	0.071	0.068
Ph_3As/CA	0.39	0.39	0.41	0.34
Ph_3Sb/CA	0.44	0.46	0.48	0.50
Ph_3Bi/CA	0.41	0.44	0.38	0.41

Polymer	**PC-3**	**MDA**	**HT-29**	**MCF-7**
Cp_2Ti/CA	0.056	0.087	0.091	0.077
Cp_2Zr/CA	0.0018	0.0022	0.0018	0.0023
Cp_2Hf/CA	0.0015	0.0011	0.0015	0.0018
Me_2Sn/CA	0.064	0.067	0.057	0.068
Et_2Sn/CA	0.081	0.043	0.039	0.052
Bu_2Sn/CA	0.074	0.051	0.097	0.048
Oc_2Sn/CA	0.056	0.047	0.044	0.062
Ph_2Sn/CA	0.055	0.064	0.059	0.053
Ph_3As/CA	0.32	0.36	0.30	0.31
Ph_3Sb/CA	0.51	0.46	0.49	0.53
Ph_3Bi/CA	0.44	0.35	0.32	0.44

Table 12 CI_{50} values calculated from data given in Table 11

	EC_{50}WI-38/ EC_{50}PANC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3
Cp_2Ti/CA	29	45	45
Cp_2Zr/CA	1000	1300	1400
Cp_2Hf/CA	1200	1500	1500
Me_2Sn/CA	0.89	0.92	0.81
Et_2Sn/CA	1.3	1.3	0.67
Bu_2Sn/CA	1.1	0.65	0.74
Oc_2Sn/CA	0.90	0.84	0.77
Ph_2Sn/CA	0.76	0.79	0.98
Ph_3As/CA	0.95	1.2	1.2
Ph_3Sb/CA	0.96	0.92	0.90
Ph_3Bi/CA	1.2	1.1	1.3

	EC_{50}WI-38/ EC_{50}MDA	EC_{50}WI-38/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}MCF-7
Cp_2Ti/CA	29	27	32
Cp_2Zr/CA	1100	1400	1100
Cp_2Hf/CA	2000	1500	1200
Me_2Sn/CA	0.81	0.95	0.79
Et_2Sn/CA	1.3	1.4	1.0
Bu_2Sn/CA	1.1	0.57	1.1
Oc_2Sn/CA	0.91	0.98	0.69
Ph_2Sn/CA	0.84	0.92	1.0
Ph_3As/CA	1.1	1.3	1.3
Ph_3Sb/CA	1.0	0.93	0.87
Ph_3Bi/CA	1.3	1.4	1.2

Figures

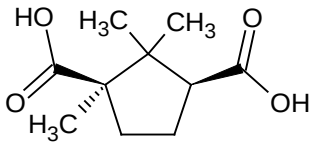


Fig. 1 D-Camphoric acid structure

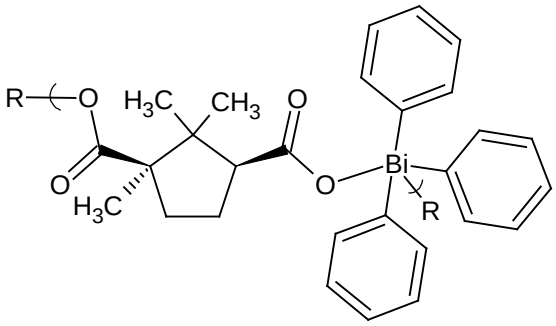
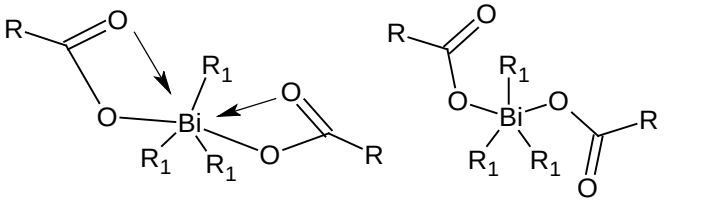


Fig. 2 Repeat unit for the product of D-camphoric acid and triphenylbismuth dichloride where R_1 represents simple chain extension



Seven bonded-bridge-like linkage

Symmetrical non-bridge-like linkage

Fig. 3 Geometrical arrangements about the metal atom

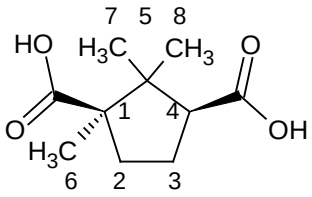


Fig 4 Structure of CA indicating positions of protons for assigned peaks

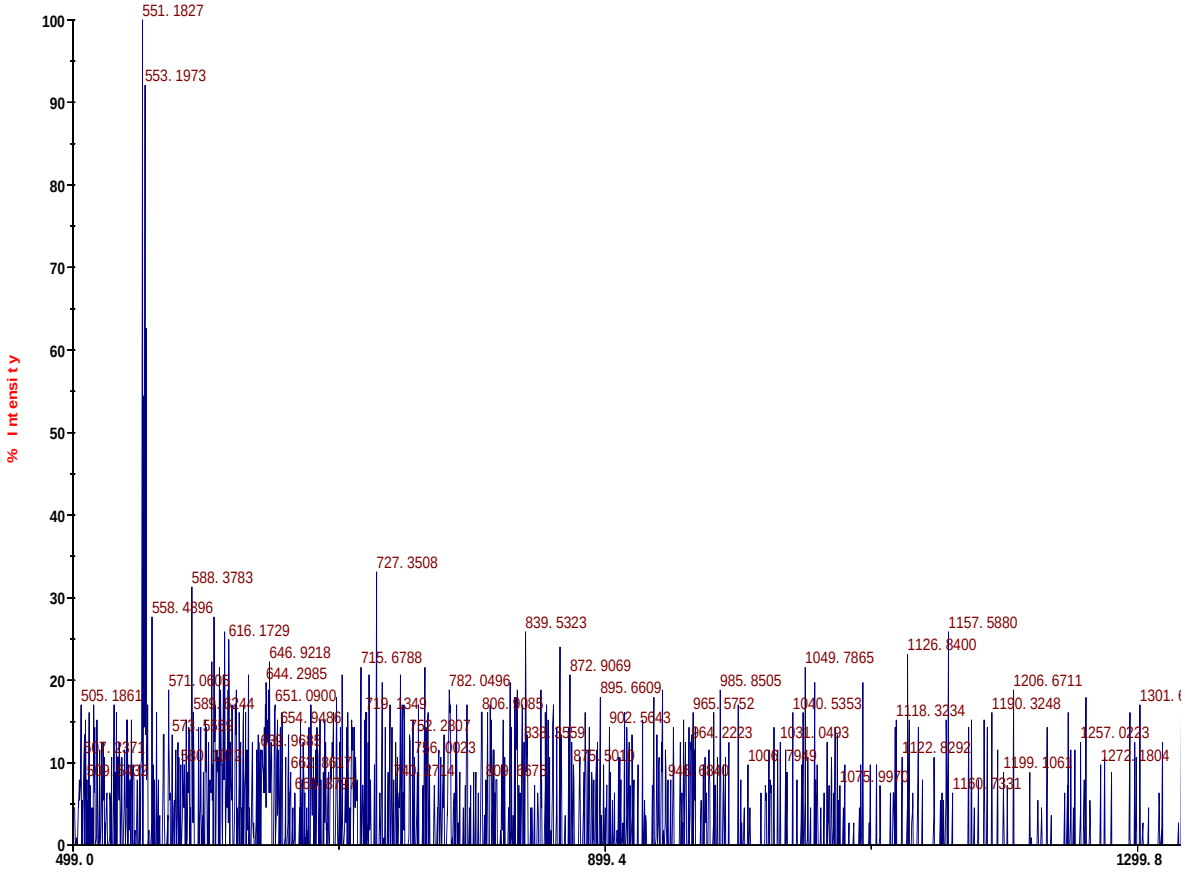


Fig 5 MALDI MS for the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 1300 Da in the reflective mode

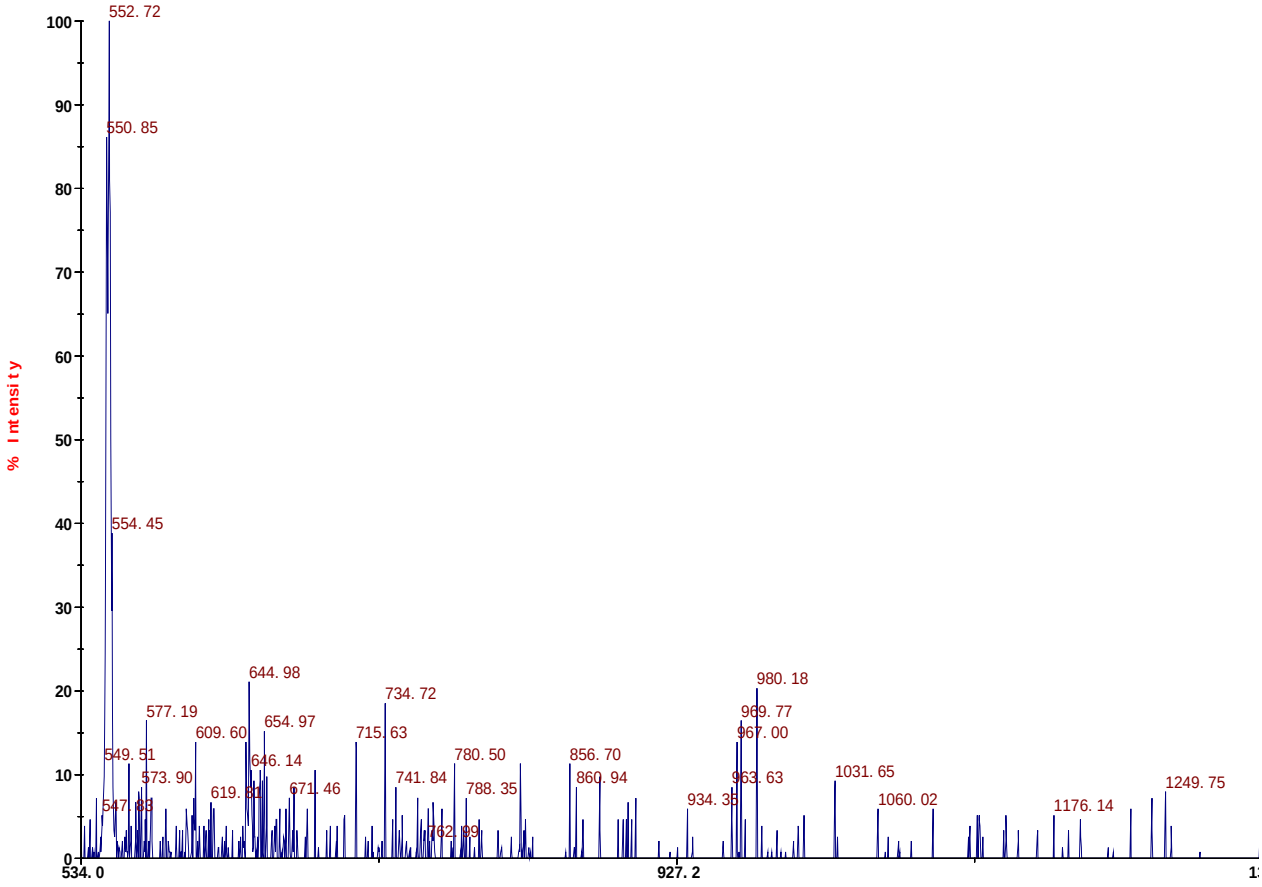


Fig. 6 MALDI MS for the product of triphenylantimony dichloride and camphoric acid over the mass range of 500 to 1300 Da in the reflective mode

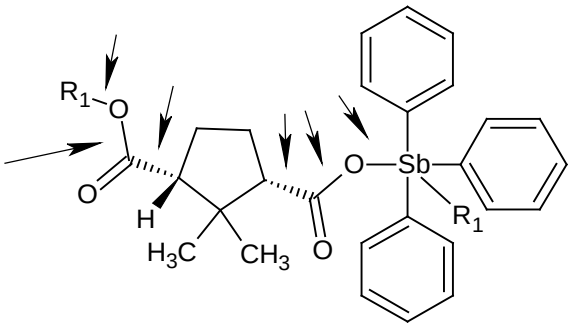


Fig. 7 Sites of preferred bond scission for the antimony polymer

CHAPTER 14

ORGANOTINS AS ANTIVIRAL AGENTS

Introduction

The current focus of organotin research in our laboratory has been aimed towards finding compounds that act as anti-microbial agents against a variety of test organisms, including bacteria and viruses. Concerning their efficacy as antiviral agents, we have tested organotin polymers derived from various antibiotics, including ampicillin and norfloxacin, polymers derived from the antiviral agent acyclovir, and polymers derived from the chemotherapeutic agent cisplatin. The results of some of these polymers as antiviral agents are described below.

Antiviral Polymers Derived from Antibiotics

Ampicillin and norfloxacin are commonly used antibiotics. Ampicillin belongs to the penicillin family of antibiotics, while norfloxacin is a fluoroquinolone antibiotic used to treat a variety of gram-positive and gram-negative bacteria that often cause urinary tract infections.[1] Multiple previous studies have demonstrated that polymers containing the dibutyltin moiety demonstrate the most antimicrobial activity, and thus, dibutyltin-containing polymers derived from ampicillin and norfloxacin were tested against a variety of viruses, including reovirus ST3, vaccinia virus, herpes simplex virus (HSV-1), and varicella zoster virus (VZV).[1] Reovirus is a double-stranded RNA virus, while the other viruses are DNA viruses. Using a standard plaque reduction assay technique for each virus, the dibutyltin-ampicillin and dibutyltin-norfloxacin polymers were tested.[1] For example, without any added polymer, the vaccinia virus at a dilution of 10^{-4} PFUs (titer: 1 x 10^{6} PFUs) gave 100 plaques.[1] The dibutyltin-ampicillin polymer at a concentration of 2 micrograms/mL reduced the number of plaques to 0.005 after 48 hours.[1]The dibutyltin-norfloxacin polymer could completely inhibit vaccinia virus infection of 143 cells at a concentration of 3 micrograms/mL.[1] Both the ampicillin and norfloxacin polymers showed good inhibition of both the RNA virus (reovirus) and the DNA viruses tested, and thus, may hold promise as future antiviral agents.[1]

Antiviral Polymers Derived from Acyclovir

Acyclovir, a nucleoside analog derived from guanosine, is frequently used in the treatment against many viral infections, particularly infections with the herpes viruses HSV-1 and HSV-2.[2] Structurally, acyclovir contains two Lewis acid-base functional groups: an alcohol group and an amine group.[2] Using acyclovir as a Lewis base, our lab created and tested a variety of organotin polyamine ethers containing acyclovir, and these polymers showed phenomenal inhibition of herpes simplex virus-1 and varicella zoster virus.[2] The various polymers showed promising results against HSV 1 and VZV, moderate inhibition of vaccinia virus, and little inhibition of reovirus ST3, a double-stranded RNA virus.[2] The dibutyltin, dimethyltin, and diphenyltin polymers showed better antiviral activity than acyclovir itself.[2]

Organotin-Derived Polymers

In continuing our efforts to find organometallic polymers that display significant antimicrobial activity, several organotin and organometallic polymers that showed promising anti-tumor activity were tested for their efficacy as antiviral agents. Increased cellular DNA replication is seen in cancerous cells, and it is also seen in most DNA and some RNA viral infections.[3] The compounds were initially tested against HSV-1, a dsDNA virus which replicates in the nucleus of the host cell, and vaccinia virus, a dsDNA virus that replicates in the cytoplasm of the host cell. Results from the initial series of organotin polyethers showed that the polymer derived from dibutyltin dichloride and ethylene glycol had high activity against vaccinia virus only, while the dibutyltin dichloride and polyethylene glycol (PEG) polymer showed good inhibition of both viruses.[3] It is believed that the promising activity of the PEG polymer towards both viruses is due to its water solubility.[3] The compounds that showed the highest antiviral activity for the organotin polyether series were the polymers derived from the aromatic diol hydroquinone.[3] All three of the tested hydroquinone-derived polymers blocked HSV-1 infection in 35 to 80% of cells, while only the dibutyltin/tert-butylhydroquinone demonstrated activity against vaccinia virus.[3] Structurally, hydroquinone possesses an aromatic ring with available pi bonds, and these pi bonds may be able to interact both extracellularly (at the point of viral attachment) and intracellularly (binding to DNA), which would explain the high antiviral activity for this series of polymers.[3]

Another group of polymers that were tested were organotin polyamines derived from the reaction of dibutyltin dichloride with 4,6-diaminopyrimidines.[3] Results showed that the polyamine derived from 4,6-

diaminopyrimidine blocked the replication of HSV-1 in in about 30% of cells, while the dibutyltin derivative of 4,6-diamino-2-mercaptopyrimidine blocks infection in 20% of treated vaccinia cells.[3] It has been surmised that the antiviral activity of these class of polymers is due to the compounds being nucleoside analogs, and the virus may incorporate the polymer into its DNA during viral replication, thereby terminating the viral DNA synthesis.[3]

Viruses Used in This Study

Vaccinia Virus

Vaccinia virus is an Orthopoxvirus within the *Poxviridae* family of viruses.[4] It is a double-stranded DNA virus that was previously used in the vaccination program against smallpox, which was eradicated through the World Health Organization (WHO) in 1979.[4] Although smallpox has been eradicated, research into the disease remains invaluable, as smallpox remains a potential agent for bioterrorism. The worldwide vaccination program against smallpox was stopped in 1980, and thus, an outbreak of smallpox today would decimate the current population who lack smallpox immunity (Riedel 2005). Smallpox is caused by two viruses: variola major and variola minor, with variola major producing most of the fatal disease burden, with a case-fatality rate of 20 percent or more (Riedel 2005). Vaccinia virus is the vaccine strain of smallpox, and although its origins are still unclear after over 100 years of research, it is surmised that vaccinia could be the product of genetic recombination (Friedel 2005).

Vaccinia virus is a unique virus because, although it is a DNA virus, it is replicated exclusively in the cytoplasm of infected cells through a viral DNA-dependent RNA polymerase (Riedel 2005). It is also unique in that vaccinia produces two types of virions: intracellular mature virus (IMV) and extracellular enveloped virus (EEV), both of which are infectious.[6] The IMV virions are surrounded by a single membrane, while the EEV virions possess two membranes and are critical in the dissemination of the virus.[6] The external membrane of the EEV virions is derived from the host cell, and thus, these virions can evade both host antibody and host complement immune responses.[6]

Upon contact with a competent host cell, vaccinia virions enter the cell through phagocytic vacuoles where they are partially stripped of their outer core, releasing the vaccinia virus dsDNA genome into the cytoplasm.[7] Once inside the cytoplasm, the DNA-dependent RNA-polymerase of the virus transcribes the vaccinia viral genome into messenger RNAs (mRNAs) (Riedel 2005). The transcribed

genes can be classified as early, intermediate, and late genes. The early vaccinia genes are needed for viral DNA replication and for assisting the virus in escaping the host's innate immune response.[6] The intermediate genes encode for transcriptional regulators of the late genes, and the late genes encode the structural proteins and enzymes necessary to construct the new virions.[6] Inside cytoplasmic inclusions known as virus factories, the vaccinia virus dsDNA is packaged into an immature virion (IV), with proteolytic cleavage leading to the formation of the intracellular mature virus (IMV), which are released through cell lysis.[6] Some virions, however, will become wrapped in Golgi-derived membranes and are known as intracellular enveloped virus (IEV).[6] These intracellular enveloped virus particles are transported to the surface of the cell, where they can either remain at the cell surface as CEV (cell-associated enveloped virus), or they are released as extracellular enveloped virus (EEV).[6]

Zika virus, an enveloped, plus-stranded RNA virus belonging to the *Flavivirus* genus, was first discovered in Uganda in 1947 (B.H. Song et al 2017). Usually, Zika virus causes asymptomatic infection; if symptoms do occur, they include a low-grade fever, itchy rash, and arthralgia (B.H. Song et al 2017). The concern for Zika virus, however, emanates from its potential to cause severe neurological disease, such as microcephaly in newborns, as well as a handful of cases which involved development of Guillain-Barre syndrome, which causes the immune system to attack the peripheral nervous system (B.H. Song et al 2017).

Zika virus particles contain an inner nucleocapsid surrounding the genomic RNA, and the nucleocapsid is wrapped in an envelope that contains the viral membrane protein (M) and the viral envelope protein (E).[8] The RNA of Zika virus encodes 3,423 amino acids which are translated as a large polyprotein which is subsequently cleaved into 10+ individual viral proteins.[8] The viral nonstructural protein NS3 has helicase and nucleoside triphosphatase activities, while NS5 is the viral RNA-dependent RNA polymerase which is required for viral genome replication.[8] Zika virus infects human neural progenitor cells (hNPCs) through clathrin-mediated endocytosis.[8] The acidic environment of the endosome induces conformational changes in the viral envelope (E) glycoprotein leading to fusion between the viral and endosomal membrane and subsequent release of the Zika virus RNA into the cellular cytoplasm.[8] The viral RNA-dependent RNA polymerase is responsible for replication and translation of the viral genome, along with currently unidentified cellular factors.[8] Immature virions bud

into the endoplasmic reticulum, where they receive their cellular-derived envelope with embedded viral prM (membrane) and envelope proteins. Immature virions complete the process of maturation as they proceed through the trans-Golgi network, and the virions are eventually released from the cell through exocytosis. [8]

Materials and Methods

Cell Culture Maintenance

The cell lines used in this study were Vero cells and 143 cells. All of the cell lines that were used are transformed cell lines with the ability to replicate indefinitely in cell culture. Vero cells are African green monkey kidney cells and support the growth of Zika virus. 143 cells, which allow for the growth of vaccinia virus, are derived from a human osteosarcoma cancer. All cells lines were maintained in 75 cm^2 flasks in Dulbecco's Modified Eagle's Media (1X MEM) with 5% fetal bovine serum (or 10% bovine growth serum) and the antibiotics streptomycin/penicillin at a final concentration of 100U/mL. All cells were kept in a 5% CO_2 incubator. The cells were subcultured as often as needed to seed new flasks or plates for experiments. Briefly, confluent cells were washed in the culture flask with 1x sodium saline citrate (SSC), or MEM without serum. Trypsin (0.05%) was then added to the cells to detach them from their flask. The trypsinized cells were placed in the 5% CO_2 incubator for 5 minutes. Following detachment, 5 mL of fresh media was added to the cells to wash them from the side of the flask. New media (up to 5 mL total for a 25 cm^2 flask and 10 mL total for a 75 cm^2 flask) was added to the flasks, usually in a 1:4 dilution (2 mL of cell suspension in 8 mL of fresh media). The cells were seeded at varying dilutions, depending on when they were next needed.

Virus Stock Preparation

Virus stocks were grown in their appropriate cell lines. Initially, cells were seeded in a 25 cm2 culture flask to 90-100 percent confluency. The following day, the cells were infected with virus. For the first passage, a multiplicity of infection (MOI) of 1 was used, which means that there was one virus particle per cell. The viral inoculum was made using diluent which consisted of 1X MEM with 100U/mL of penicillin/streptomycin and no growth serum. After one hour of virus adsorption with rocking every 15

minutes, 5 mL of 1X MEM with 5% BCS and 1% P/S was added. When the virus had killed 50% of the infected cells, which was seen microscopically as a cytopathic effect (CPE), the cells were sonicated to release all of the virus particles from the cells. Upon second passage, 1 mL of the passage 1 viral lysate was used to infect a confluent 75 cm^2 flask. The third and final passage involved infecting cells in a 150 cm^2 flask with 2 mL of the second viral passage. The third passage is the viral passage that was titered.

Viral Titration

To determine the titer of each virus stock in PFU/mL, standard plaque assays were used. Initially, the virus was serially passaged three times in a tissue culture of the appropriate cell line (vaccinia: 143; Zika: Vero), first in a 25 cm^2 flask, followed by a 75 cm^2 and then a 150 cm^2 flask. The cells in the 150 flask cm^2 were then sonicated to lyse the cells and release the virus. The third passage lysate was then used to infect confluent monolayers of cells for a plaque assay. Vero cells were seeded into 6-well plates for Zika virus and 143 cells were seeded into 12-well plates for vaccinia virus in 1x MEM containing 5% fetal bovine serum (FBS) and penicillin/streptomycin at the concentration seen above. When the cells reached around 80 percent confluency ~24 hours later, the media was aspirated off, and the cells were infected with serial dilutions (10^{-1} to 10^{-7}) of virus at an inoculum size of 250 microliters (6-well plate) or 125 microliters (12-well plate). To obtain serial dilutions, 100 microliters of the third passage viral lysate was suspended in 900 microliters of diluent, which is 1x MEM with no FBS, and 1% penicillin/streptomycin. This yields a 10^{-1} dilution. This dilution was then vortexed, and 100 microliters of this dilution was then placed into another tube containing 900 microliters of diluent. The process was repeated until all desired dilutions had been created. The control for each plate contained cells and 250 microliters (or 125 uL) of diluent without added virus. Following one hour of incubation with agitation every 15 minutes**,** the cells were washed, and fresh media was added to each well. After the assays were incubated (2 days for vaccinia, 3 days for Zika), both were stained with 1% crystal violet in 50% ethanol.

Vaccinia Titer: 822 plaque in ½ mL = 1644 x 10^{4} PFU/mL

= 1.644 x 10^{7} PFU/mL

Compound Preparation

All compounds arrived to us in powdered form from the laboratory of Dr. Charles Carraher at Florida Atlantic University. Initially, 0.01 g (10 mg) of compound was dissolved in 1 mL of DMSO, giving a stock concentration of 10 mg/mL. One microliter of the stock solution was then placed into 1 mL of AquaPure sterile water, giving a concentration of 10 microgram/mL. Finally, 1 mL of this solution was placed into 10 mL of AquaPure water, giving a beginning concentration of 1 microgram/mL for compound testing.

Cytotoxicity Assays

To determine the highest concentration of compound that the cell lines could tolerate without showing cytopathic effects, cytotoxicity assays were performed for each compound. For each of the three cell lines, 125,000 cells were seeded in 500 microliters of media in each well of a 24 well plate. When the cells reached confluency around 24 hours later, compounds were added to the wells. The 1 microgram/mL starting concentration for each compound was ten-fold serially diluted in 1x MEM, yielding $10^{\wedge-1}$ to $10^{\wedge-4}$ compound dilutions. The media from each well of the 24 well plates were removed, and 500 microliters of the compound dilutions were added to each well. After 96 hours, 50 microliters of trypan blue were added to each well and allowed to stain the cells for 5 minutes. After 5 minutes, the cells were observed microscopically to determine the highest concentration of drug for each cell line that did not cause cell death.

Viral Plaque Reduction Assays

Vaccinia Assay

For the vaccinia plaque reduction assays, 12 well plates were used. When the cells had reached confluency, the media was removed, and vaccinia virus at a 10^-4 dilution was added to each test well. For control wells, only 1x MEM was added. Control wells of DMSO dilutions were also completed in which the DMSO was diluted in MEM as if it were a drug. All wells, whether control or test wells, received a 125-microliter inoculum. After 1 hour of virus adsorption with agitation every 15 minutes, the cells were washed with 1X MEM without serum to remove any remaining virus. The compounds were then added to

the wells, 1 mL per well, at the concentrations previously determined by the cytotoxicity assays. After 48 hours, the old media was removed, and 1% crystal violet was used to stain the cells.

Zika Assay

For the zika virus assay, 96-well plates were used. After the cells reached confluency in their wells ~24 hours later, the old media was removed from each well and 100 microliters of fresh media was added. For each compound, 200 microliters of drug were added to the first well of each column. After mixing the solution, 100 microliters were taken from the first well and placed into the second well. The compounds were serially two-fold diluted across the 96-well plates. Following the addition of the compounds, 10 microliters of Zika virus was added to the test wells. The assays were placed in the incubator for 72 hours and then viewed microscopically. The lowest concentration of compound that prevented the Zika-infected cells from showing cytopathic effects (CPE) was recorded.

Results

Table 1 Group II Compounds Vaccinia Plaque Reduction Assay Results: Diorganotins + Camphoric Acid

Compound ID	Drug ug/mL	% Virus Produced	% Virus Produced	Avg. % Virus Produced	Avg. Inhibition %	Standard Deviation	Significance at p < 0.05
AC3	0.1	106.613	97.395	102.004	-2.004	6.518	No
AC4	0.1	72.144	75.351	73.746	26.253	2.268	No
AC5	0.1	112.224	117.034	114.629	-14.629	3.041	No
Acyclovir	1.0	0	0	0	100	-	-
No drug	-	100	100	100	0	-	-

Table 2 Group III Compounds Vaccinia Virus: 3-AT Derivatives

Compound ID	Drug ug/mL	% Virus Produced	% Virus Produced	Avg. Virus Produced	Avg. Inhibition %	Standard Deviation	Significance at p < 0.05
RC1	0.1	95.822	73.035	84.429	15.572	16.113	No
RC2	0.1	73.321	82.961	78.141	21.859	6.817	Yes
RC3	0.1	80.916	85.006	82.961	17.039	2.892	Yes
RC4	0.1	89.103	81.215	85.159	14.841	5.578	No
RC5	0.1	65.141	64.557	64.849	35.151	0.413	Yes
Acyclovir	1.0	0	0	0	100	-	-
No drug	-	100	100	100	0	-	-

Table 3 Group IV Compounds Vaccinia Plaque Reduction Assay Results

Compound ID	Drug ug/mL	% Virus Produced	% Virus Produced	Avg. % Virus Produced	Avg. Inhibition %	Standard Deviation	Significance at p < 0.05
FM1	0.1	126.653	105.411	116.032	-16.032	15.020	No
FM3	0.1	114.228	137.876	126.052	-26.052	16.722	No
FM5	0.1	106.212	91.784	98.998	1.002	10.202	No
Camphoric acid	0.1	123.273	129.992126.634	126.634	-26.634	4.751	Yes
Acyclovir	1.0	0	0	0	100	-	-
No drug	-	100	100	100	0	-	-

Table 4 Group V Compounds Vaccinia Plaque Reduction Assay Results: Organometallics + dicumarol

Compound ID	Drug ug/mL	% Virus Produced	% Virus Produced	Avg. % Virus Produced	Avg. Inhibition %	Standard Deviation	Significance at p < 0.05
NS1	0.001	121.265	124.429	122.847	-22.847	2.237	YES
NS2	0.001	124.429	139.543	131.986	-31.986	10.687	YES
NS3	0.001	121.968	130.053	126.011	-26.011	5.717	YES
NS4	0.001	168.366	159.930	164.148	-64.148	5.965	YES
NS5	0.001	151.845	150.791	151.318	-51.318	0.745	YES
NS6	0.001	153.251	143.761	148.506	-48.406	6.710	YES
NS7	0.001	132.513	152.548	142.531	-42.5305	14.167	YES
Dicumarol	0.001	135.325	135.325	135.325	-35.325	0.00	YES
Acyclovir	1.0	0	0	0	100	-	-
No drug	-	100.00	100.00	100.00	0.00	-	-

Table 5 Vaccinia Plaque Reduction Assay Results: Groups I, VI, VIID

Compound ID	Drug ug/mL	Polymer	% Virus Produced	% Virus Produced	Avg. Virus Produced	Avg. Inhibition %	Standard Deviation	Significant at p < 0.05
AB1	0.0001	Bu_2Sn derivative*	88.936	85.957	87.447	12.554	2.106	Yes
ME2	0.01	Ph_2Sn PEG 8000	94.468	89.362	91.925	8.085	3.610	No
ME3	0.01	Ph_2Sn PEG 2000 Lowest mol. Wt.	112.766	114.468	113.617	-13.617	1.203	Yes
ME4	0.01	Ph_2Sn PEG 2000 medium mol. Wt.	137.449	122.553	130.001	-30.001	10.533	No
VS2	0.001	Me_2Sn and CHA	121.702	113.617	117.660	-17.66	5.717	No
VS3	0.001	Et_2Sn and CHA	100.426	103.404	101.915	-1.915	2.106	No
CHA	0.001	Monomer	167.397	130.879	149.138	-49.138	25.822	Yes
Bu_2SnCl_2	0.001	Monomer	95.015; 88.856	89.736	91.232	8.768	3.383	Yes
Acyclovir	1.0	Antiviral	0	0	0	100	-	-
No drug	-	Control	100	100	100	0	-	-

*Derivative of Bu_2Sn and 4,6-diamino-5-isoamyl-2-(3-phenyl propylamino) pyrimidine

Table 6 Vaccinia Plaque Reduction Assay Results Group VIIA Compounds

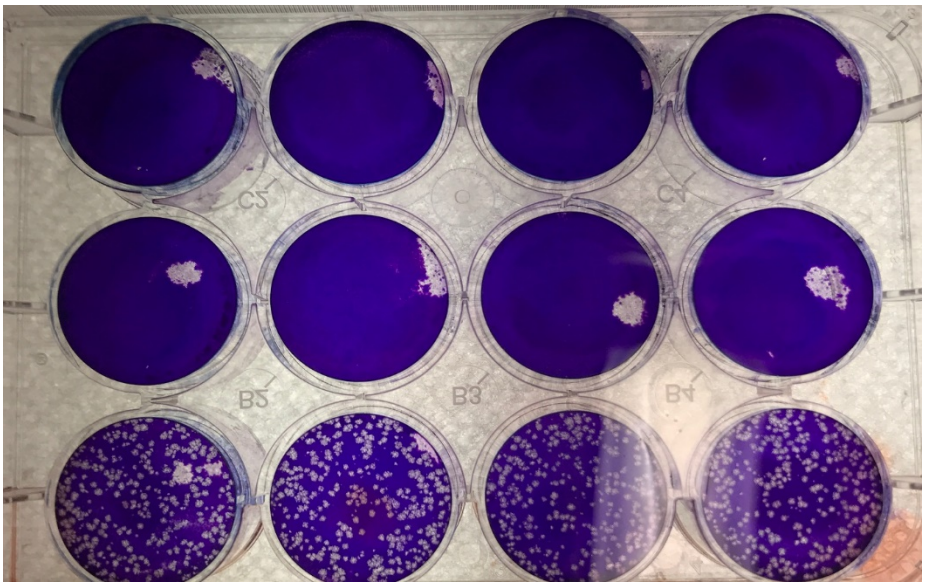
Compound ID	Drug ug/mL	Polymer	% Virus Produced	% Virus Produced	% Virus Produced	Avg. Virus Produced	Avg. Inhibition	Standard Deviation	Significant at p < 0.05
PEG 400	0.01	Cp_2Ti/PEG 400	102.053	89.736	117.889	103.226	-3.226	14.113	No
PEG 3400	0.01	Cp_2Ti/PEG 3400	101.173	91.495	91.495	94.721	5.729	5.588	No
Acyclovir	1.0	Antiviral	0	0	0	0	100	-	-
No drug	-	Control	100	100	100	100	0	-	-

Table 7 Results Cytopathic Effect (CPE) Zika Virus

Zika Strain	Compound ID	Polymer	Concentration for 100% Virus Inhibition ug/mL
Zika 501	KB5	Cp_2HF/PEG 400	0.5
Zika 501	KB8	Cp_2Hf/PEG 8000	0.5
Zika 501	FM1	Ph_3Sb/Camphoric Acid	0.5
Zika 501	FM5	Me_2Sn/*Lamivudine	0.025
Zika 501	AC3	Me_2Sn/Camphoric acid	> 0.1
Zika 501	AC4	Et_2Sn/Camphoric acid	0.025
Zika 501	AC5	Ph_2Sn/Camphoric acid	0.025
Zika 502	AC3	Me_2Sn/Camphoric acid	> 0.1
Zika 502	AC4	Et_2Sn/Camphoric acid	0.000391
Zika 502	AC5	Ph_2Sn/Camphoric acid	0.000781
Zika 502	FM5	Me_2Sn/*Lamivudine	> 0.1

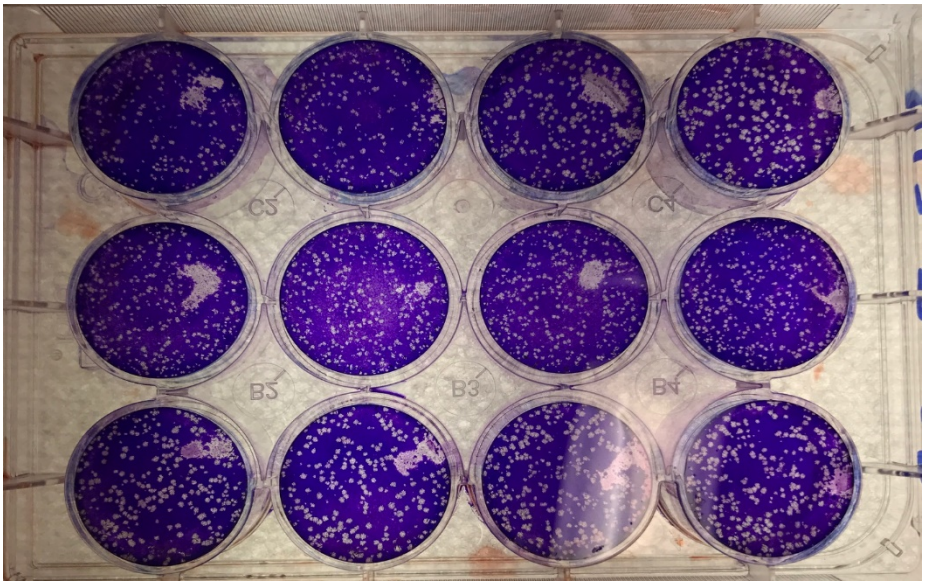
*Lamivudine is an anti-retroviral drug used in the treatment of HIV.

Figure 1: Vaccinia Plaque Reduction Assay Control Plate



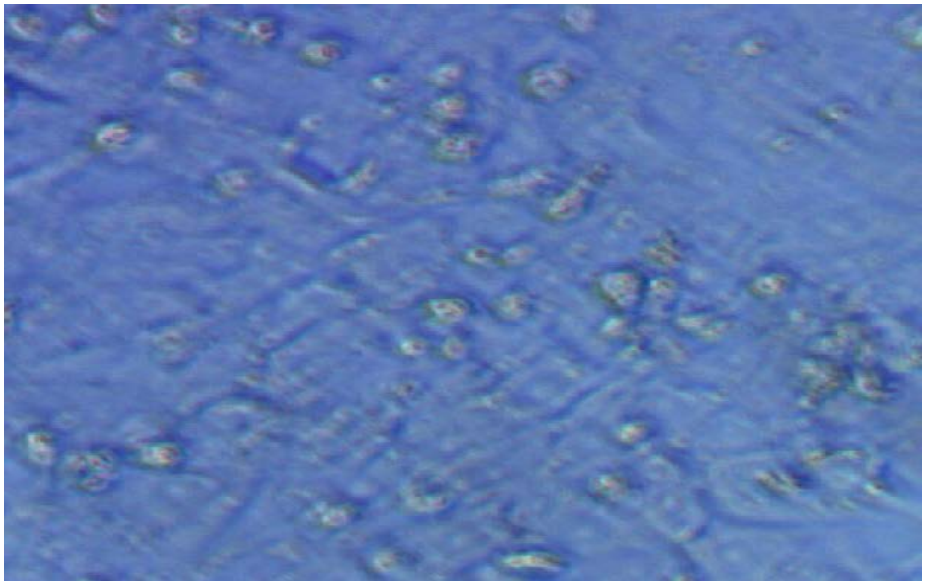
Row A: 143 cells stained with 1% crystal violet in 50% EtOH. There are no areas of white plaques, indicating living cells; **Row B:** Acyclovir positive controls. These cells were infected with vaccinia and then the antiviral drug acyclovir was added. No viral plaques are seen, as acyclovir prevents viral replication; **Row C**: Vaccinia-infected 143 controls at 10^{-4} PFU/mL. Numerous plaques are seen on the 143 cells where the virus has caused cell lysis.

Figure 2: Vaccinia Plaque Assay Compound Test Plate



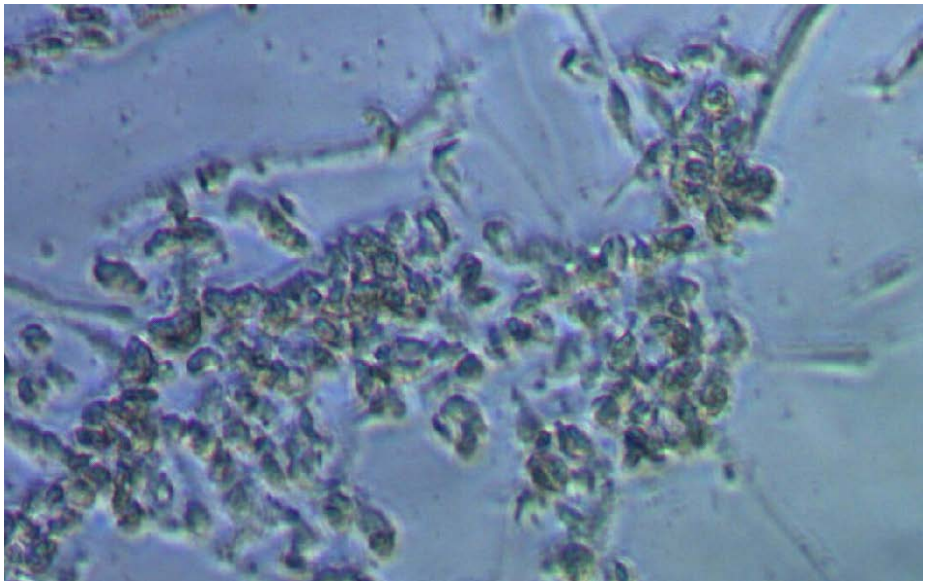
Row A: Compounds ME2, AB1 in duplicate; Row B: Compounds ME4, ME3 in duplicate, Row C: Compounds VS3, VS2 in duplicate. These look similar to the virus infected cells seen in row C of figure 2. This indicates that these compounds did not show a significant reduction in vaccinia activity.

Figure 3 Zika Virus Control Non-Infected Vero Cells (Living)



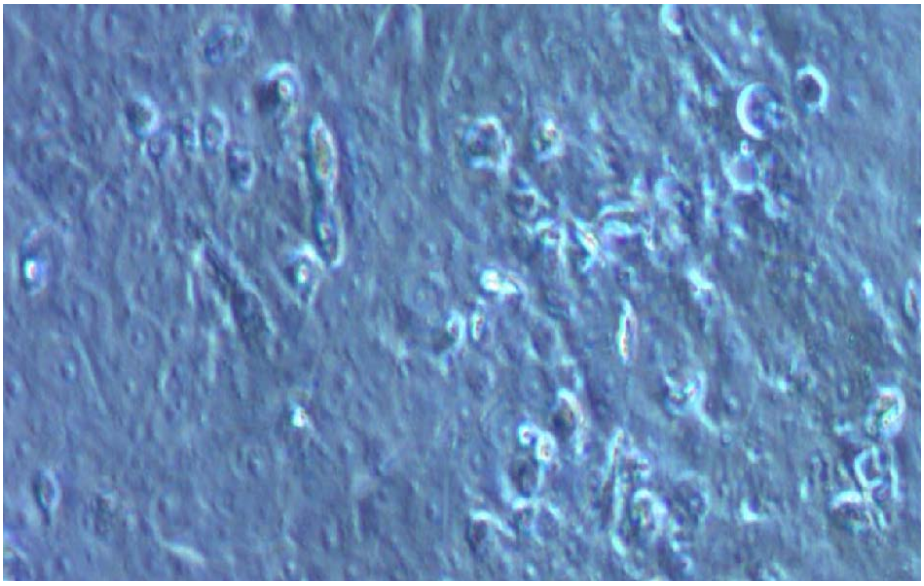
This figure depicts living Vero cells. The rounded cells are actively dividing, while the more elongated cells are adherent to the culture flask.

Figure 4 Zika-Infected Vero Cells (Dead)



Zika-virus infected Vero cells. The cells are clumped together and are dead.

Figure 5 Zika-Infected Vero Cells with Compound AC4 (Living)



Zika-virus infected Vero cells. These cells were treated with compound AC4 at the time of infection. They are living cells that look like the controls cells seen in figure 3, indicating that the compound has reduced the ability of Zika virus to replicate.

References

1) Roner, Michael R., Charles E. Carraher Jr., Joann L. Roehr, Kelly D. Bassett, and Deborah W. Siegmann-Louda. "Anti-Viral Activity of Norfloxacin and Ampicillin and Dibutyltin Polymers Derived From Norfloxacin and Ampicillin Against Reovirus ST3, Vaccinia Virus, Herpes Simplex Virus (HSV-1) And Varicella Zoster Virus (VSV)." (2004): n. pag

2) Carraher, Charles E., Theodore S. Sabir, Michael R. Roner, Kimberely Shahi, Robert E. Bleicher, Joanne L. Roehr, and Kelly D. Bassett. "Synthesis of Organotin Polyamine Ethers Containing Acyclovir and Their Preliminary Anticancer and Antiviral Activity."Journal of Inorganic and Organometallic Polymers and Materials 16.3 (2006): 249-57.

3) Roner, Michael R., Charles E. Carraher Jr., Kimberly Shahi, and Girish Barot. "Antiviral Activity of Metal-Containing Polymers—Organotin and Cisplatin-Like Polymers." Materials 4.12 (2011): 991-1012

4) Verardi, Paulo H., Allison Titong, and Caitlin J. Hagen. "A Vaccinia Virus Renaissance."Human Vaccines & Immunotherapeutics 8.7 (2012): 961-70.

5) Riedel, Stefan, MD, PhD. "Smallpox and Biological Warfare: A Disease Revisited." Baylor University Medical Center Proceedings 18.1 (2005): 13-20.

6) Roberts, Kim L., and Geoffrey L. Smith. "Vaccinia Virus Morphogenesis and Dissemination."Trends in Microbiology 16.10 (2008): 472-79

7) Joklik, W.K., D. Phil. "Virus Synthesis and Replication: Reovirus vs. Vaccinia Virus." The Yale Journal of Biology and Medicine 53 (1980): 27-39

8) Song, Byung-Hak, Sang-Im Yun, Michael Woolley Woolley, and Young-Min Lee. "Zika Virus: History, Epidemiology, Transmission, and Clinical Presentation." Journal of Neuroimmunology (2017): 1-15.

CHAPTER 15

CONCLUSIONS

Future of Organometallic Polymers as Anti-Cancer Agents

For many years, our laboratory has been involved in testing many organometallic polymers for their efficacy as anti-cancer agents. By combining tin or another organometallic agent with a biologically active Lewis base, we have found that there appears to be a synergistic effect, with the polymer typically outperforming either of the individual reactants in the compound. We have found several compounds that outperform cisplatin, one of the most widely utilized chemotherapeutic agents. These compounds have been active against a multitude of cancer types, including breast, prostate, bone, lung, colon, and most importantly, pancreatic cancer. Pancreatic cancer is inevitably fatal, as the cancer has usually metastasized throughout the body at the time of diagnosis. As such, we place strong emphasis on the ability of our compounds to inhibit the PANC-1 and AsPC-1 pancreatic cancer cell lines. When examining the efficacy of our compounds, we often look at two measures of cell inhibition: the effective concentration, and the chemotherapeutic index. A lower number is preferred for the effective concentration when examining cancerous cell lines, as this indicates that the drug is highly toxic at low doses to the cancer cell lines. In contrast, higher numbers are preferred for the chemotherapeutic index. Values over 2 are considered significant and indicate that a compound preferentially kills cancerous cells over normal cells.

Group VI compounds are derived from various organotin dihalides and alpha-4-cyanohydroxycinnamic acid (CHA). **Table 5** in chapter 2 shows the results for these CHA derivatives against a variety of cancer cell lines, however, the most significant findings were against the pancreatic cancer cell lines. For both the PANC-1 and AsPC-1 cell lines, the effective concentration for cisplatin is much higher than the effective concentration for the CHA-derived polymers. Simple organotin polyethers created from simple, non-inhibitory diols also showed a significant ability to inhibit the growth of pancreatic cancer cells. Thus, organotin and CHA derivatives, along with organotin and simple diol derivatives could show promise in the future as novel chemotherapeutic agents against pancreatic cancer.

Another group of polymers that showed significant anti-cancer ability were the polymers derived from the group IVB metallocenes and CHA. As can be seen in **publication 8**, these polymers showed effective concentrations that were much lower than the standard, cisplatin. The most promising compounds contained the metallocenes hafnocene or zirconocene, so these compounds also show promise for future use in cancer treatment. Furthermore, as depicted in chapter 2 **table 6**, polymers derived from titanocene and various polyethylene glycols (PEGs) inhibited a variety of cancer cell lines within the same range as cisplatin, particularly for the two pancreatic cancer cell lines. The group IVB metallocene-derived polymers appear to show the most promise for future anti-cancer activity.

Future of Organometallic Polymers as Antiviral Agents

Due to the success of a variety of organometallic polymers as anticancer agents, we tested a number of these compounds for their efficacy as antiviral agents. Although the results of the present study did not show incredible viral inhibition, there are a few compounds which show promising activity against vaccinia virus (dsDNA) and Zika virus (+RNA).

Vaccinia virus is a dsDNA virus with a unique intracytoplasmic replication mechanism. Although smallpox has been eradicated worldwide, vaccinia remains relevant because of its potential use in biowarfare. Using standard plaque reduction assays, several organometallic polymers were tested against vaccinia virus for their ability to inhibit viral replication in 143 cells. Most polymers were not active; however, the derivatives of 3-AT (**table 3** of chapter 3) showed some inhibition, protecting between 15 and 35 percent of cells from infection. These compounds warrant retesting. Also, although it appears that many of the tested compounds seemed to enhance the average virus produced, this is easily explained. In **table 4** of chapter 3, for example, all the organometallic/dicumarol polymers showed over 100% virus production. In this case, and in the case of other groups of polymers, the compounds made the cells more susceptible to lysis, resulting in an enhanced production of visible plaques compared to the number of visible plaques for the virus-infected control wells. Thus, in these instances, there was not an increase in virus production, but an increased in susceptible cells.

Zika virus is a single-stranded, plus-sense RNA virus that has garnered worldwide attention recently due to its connection to neurological birth defects. **Table 7** shows the results for the compound assays for Zika virus, while **figures 3, 4** and **5** show pictures of some of the Zika virus CPE assays.

Unlike vaccinia virus, in which a plaque assay technique was used to assess the efficacy of the compounds, we could not get Zika virus to produce defined plaques, so we used a cytopathic effect assay. My results indicate that three groups of compounds show promise as antiviral agents against Zika virus: group II compounds (AC3, AC4, AC5), group IV compounds (FM1, FM5), and group VIIC compounds (KB5, KB8). Against Zika strain 501, AC4 (ethyltin/camphoric acid), AC5 (phenyltin/camphoric acid), and FM5 (triphenyl/camphoric acid derivative) all inhibited infection of Vero cells at 0.025 microgram/mL. Against Zika strain 502, AC4 and AC5 inhibited infection of Vero cells at concentrations of 0.000391 and 0.000781 micrograms/mL, respectively. Thus, it appears that the organotin and camphoric acid-derived compounds merit further testing against Zika virus as potential novel antiviral agents.