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COMPARATIVE INHIBITION OF CANCER CELL LINES BY THE SYNTHETIC THYMIDING NUCLEIC ACIDS AND GROUP 4 METALLOCENE DICHLORIDES AND ORGANOTIN DIHALIDES

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ABSTRACT

The ability to inhibit cancer cell lines by two groups of synthetic nucleic acids derived from thymidine and group 4 metallocenes and organotin dihalides was evaluated. Overall, the group 4 metallocene synthetic nucleic acids showed superior ability to inhibit the cell lines compared with the organotin dihalides with good inhibit in the nanogram/mL range. Within the group 4 metallocene polymers, the order of inhibition was Zr>Hf>Ti. Thus, for this group of polymers the zirconocene polymers show the greatest ability to inhibit cancer cell lines.

KEYWORDS: thymidine, organotin dihalides, organotin polymers, synthetic nucleic acids, metallocene polymers, pancreatic cancer, breast cancer, glioblastomas brain cancer, interfacial polymerization.

INTRODUCTION

Carraher and Millich were issued the first patent for the chemical synthesis of nucleic acids in 1971.[1] The nucleic acid was derived from thymidine (2-deoxy-D-ribose) and phosphorus dichlorides. We selected thymidine since it has only two active functional groups, alcohols. Thymidine, (Figure 1), is one of the four bases present in DNA and RNA. Since thymidine has only two reactive groups, the polymerization products are liner rather than crosslinked. The other natural nucleic acids contain additional functional groups so complicate the synthesis of linear products.

This was followed by the synthesis of additional related nucleic acid like polymers.[2-7]

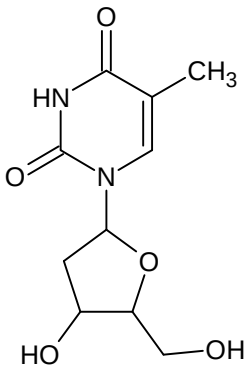

Figure 01: Thymidine structure.

One of the major purposes for our recent activity is to look at the factors that influence the ability to inhibit cancer. As part of this effort this paper is one of our first to look at the influence of the nature of the metal on inhibition of cancer growth. In this paper we look the relative influence of group 4 metallocenes and organotin moieties on cancer inhibition.

Recently, we described work on thymidine synthetic nucleic acids derived from metallocene dichloride (Figure 1) [8] and organotin dihalides (Figure 2).[9] Here we compare the ability of these two groups of polymers on their ability to inhibit cancer growth.

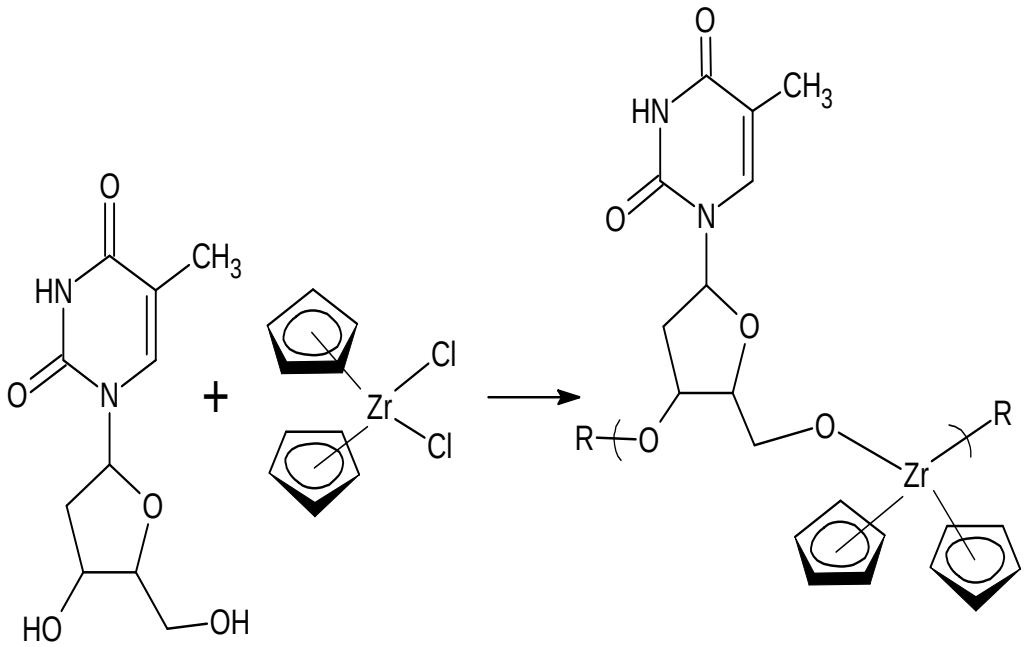

Figure 02: General repeat unit for the product of zirconocene dichloride and thymidine where R represents simple chain extension.

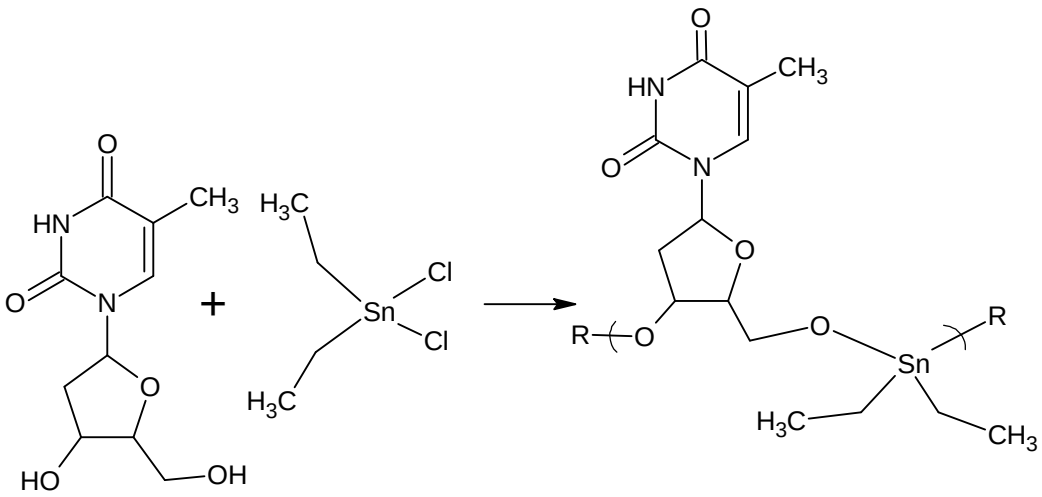

Figure 03: General representation for the repeat unit of the product of diethyltin dichloride and thymidine where R represents simple chain extension.

Experimental

Reactions and Reactants: Reactions were carried out using the interfacial polycondensation technique. Briefly, an aqueous solution (30 ml) containing the thymidine (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 room temperature, at about 25^{o}C). Stirring was begun and a chloroform or hexane solution (30 ml) containing the metallocene dichloride (0.00300 mol) or organotin dichlorides (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 or hexane to remove unreacted materials and unwanted by-products. The solid was washed onto a glass petri dish and allowed to dry at room temperature.

Diphenyltin dichloride (1135-99-5), dimethyltin dichloride (753-73-1), thymidine (50-89-5) and dibutyltin dichloride (683-18-1) were purchased from Aldrich Chemical Co., Milwaukee, WS; diethyltin dichloride (866-55-7) was obtained from Peninsular Chemical Res., Gainesville, FL; dioctyltin dichloride (3542-36-7) was obtained from Ventron Alfa Inorganics, Beverly, Mass. Titanocene dichloride (1271-19-8), and zirconocene dichloride (1291-32-3) were purchased from Aldrich Chemical Co., Milwaukee, WS; and hafnocene dichloride (12116-66-4) was obtained from Ventron Alfa Inorganics, Beverly, Mass. The reactants were used as received.

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) cell line was employed as the standard. Following a 24 hr 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 hrs. Following incubation, Cell Titer-Blue reagent (Promega Corporation) was added (20 uL/well) and incubated for 2 hrs. Fluorescence was determined at 530/590 nm and converted to % cell viability versus control cells.

All cytotoxicity values were 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 in Eagle's minimal essential medium, MEM, to treat the cells, then the mock-treated cells were "treated" with the same serial dilutions of DMSO without added chemotherapeutic compound. This was done to ensure that any observed cytotoxicity 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. When inhibition begins, the slop of the concentration/inhibition curve is steep until total inhibition occurs.

Cell inhibition results

Much of our recent effort is the synthesis of potential drugs for the purpose of evaluation of structure/cancer activity building a catalogue of results that will assist in further syntheses. The cancer cells employed in the current study are given in Table 1.

Table 01: 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
	U251	Human	Glioblastoma multiforme	Astrocytoma
	G55	Human	Glioblastoma	Astrocytoma
	AsPC-1	Human	Pancreatic cells	Adenocarcinoma
	PANC-1	Human	Epithelioid pancreatic cells	Carcinoma

There are two major values used to evaluate the effectiveness of materials ability to inhibit cell growth. The first is the amount of material required to inhibit cell growth to some degree, typically 50%. The term effective concentration (EC) will be employed to describe this. Tables 2 and 3 contain EC_{50} results for the polymers considered in the present study.

The EC_{50} values for the metallocene polymers are generally a decade lower compared with the organotin polymers. For the metallocene polymers that order is Ti>Hf>Zr with the lowest values in the nanogram/mL range and among the lowest for the pancreatic cancer cells. Thus, based on the EC_{50} values the zirconocene products exhibit the lowest values offering the best inhibition of the cancer cell lines.

The second value typically used to evaluate the effectiveness of materials at inhibiting the cancer cell lines is the ratio of the EC_{50} for a standard compared with the EC_{50} for the particular cell line, namely the particular values given in Tables 2 and 3. The WI-38 is generally taken as the standard in these measures. Large values are desired since they indicate a differentiation between the ability for the compound to inhibit the cancer cell lines compared to the standard cell line. These values are referred to as the chemotherapeutic index, CI or for the EC_{50} values the CI_{50}. Tables 4 and 5 contain the EC_{50} values for the compounds considered in the present study.

Table 02: EC_{50} Concentrations (micrograms/mL) for the tested thymidine metallocene compounds. Values given in ( ) are standard deviations for each set of measurements.

Sample	WI-38	PANC-1	AsPC-1	PC3
Cp_2Ti/TH	0.041(.005)	0.026(.003)	0.028(.003)	0.039(.003)
Cp_2Zr/ TH	0.046(.005)	0.0070(.003)	0.0070(.005)	0.0050(.003)
Cp_2Hf/ TH	0.043(.005)	0.022(.003)	0.014(.003)	0.047(.003)
Thymidine	1.7(.5)	1.7(.5)	1.8(.5)	1.8(.05)
Cisplatin	0.019(.01)	.0023(.005)	.0035(.005)	0.0044(.004)

Sample	MDA-MB	HT-29	MCF-7	U251	G56
Cp_2Ti/ TH	0.036(.003)	0.024(.003)	0.031(.003)	14(,07)	120(.07)
Cp_2Zr/ TH	0.0090(.03)	0.0040(.0003)	0.0040(.0003)	0.026(.03)	0.035(.03)
Cp_2Hf/ TH	0.044(.003)	0.029(.003)	0.032(.003)	0.062(.03)	0.071(.03)
Thymidine	1.8(.05)	1.8(.05)	1.8(0.5)	1.7(.5)	1.8(.05)
Cisplatin	0.0029(.002)	0.0041(.003)	0.0057(.003)	0.015(.01)	0.021(.01)

Table 03: EC_{50} Concentrations (micrograms/mL) for the organotin polymers. Values Given in ( ) are standard deviations for each set of measurements.

Sample	*WI-38*	*PANC-1*	*AsPC-1*	*U251*	*G55*
Me_2Sn/TH	0.45(.05)	0.25(.02)	0.23(.02)	0.51(.04)	0.55(.04)
Et_2Sn/TH	0.45(.05)	0.13(.02)	0.12(.02)	0.23(.04)	0.28(.04)
Bu_2Sn/TH	0.46(.05)	0.24(.02)	0.22(.02)	0.42(.04)	0.44(.04)
Oc_2Sn/TH	0.44(.05)	0.23(.02)	0.22(.02)	0.44(.04)	0.49(.04)
Ph_2Sn/TH	0.43(.05)	0.20(.02)	0.22(.02)	0.35(.04)	0.37(.04)
Thymidine	1.7(.5)	1.7(.5)	1.8(.5)	1.8(.5)	1.8(.5)
Cisplatin	0.012(.01)	0.0023(.005)	0.0035(.005)	0.015(.01)	0.020(.01)

Sample	*PC-3*	*MDA-MB-231*	*HT-29*	*MCF-7*
Me_2Sn/TH	0.27(.02)	0.24(.02)	0.22(.02)	0.22(.02)
Et_2Sn/TH	0.13(.02)	0.11(.02)	0.12(.02)	0.11(.02)
Bu_2Sn/TH	0.23(.02)	0.25(.02)	0.21(.02)	0.23(.02)
Oc_2Sn/TH	0.21(.02)	0.22(.02)	0.21(.02)	0.22(.02)
Ph_2Sn/TH	0.24(.02)	0.24(.02)	0.21(.02)	0.21(.02)
Thymidine	1.8(.05)	1.8(.05)	1.8(0.5)	1.7(.5)
Cisplatin	0.0044(.004)	0.0029(.002)	0.0041(.003)	0.0057(.003)

Table 04: CI_{50} Values for the thymidine/metallocene compounds.

Sample	EC_{50}WI-38/ EC_{50}PNC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}MDA
Cp_2Ti/ TH	1.6	1.5	1.1	1.1
Cp_2Zr/ TH	6.6	6.6	9.2	5.1
Cp_2Hf/ TH	2.0	3.1	0.92	1.0
Cisplatin	8.3	5.4	4.4	6.6

Sample	EC_{50} *WI-38*/ EC_{50}HT-29	EC_{50} *WI-38*/ EC_{50}MCF-7	EC_{50} *WI-38*/ EC_{50}*U251*	EC_{50} *WI-38*/ EC_{50}*G55*
Cp_2Ti/ TH	1.7	1.3	0.003	0.0003
Cp_2Zr/ TH	12	12	1.8	1.3
Cp_2Hf/ TH	1.5	1.3	0.70	0.61
Cisplatin	4.6	3.3	0.80	0.57

Table 05: CI_{50} values for thymidine/organotin polymers.

Sample	EC_{50}WI-38/ EC_{50}PNC-1	EC_{50}WI-38/ EC_{50}AsPC-1	EC_{50}WI-38/ EC_{50}PC-3	EC_{50}WI-38/ EC_{50}MDA
Me_2Sn/TH	1.8	2.0	1.7	1.9
Et_2Sn/TH	3.5	3.8	3.5	2.8
Bu_2Sn/TH	1.9	2.1	2.3	1.8
Oc_2Sn/TH	1.9	1.9	2.1	2.0
Ph_2Sn/TH	2.2	2.0	1.8	1.8
Cisplatin	5.2	3.4	2.7	4.1

Sample	EC_{50}WI-38/ EC_{50}U251	EC_{50}WI-38/ EC_{50}G55	EC_{50}WI-38/ EC_{50}HT-29	EC_{50}WI-38/ EC_{50}MCF-7
Me_2Sn/TH	0.88	0.82	2.1	2.1
Et_2Sn/TH	0.20	0.16	3.8	4.1
Bu_2Sn/TH	1.0	1.2	2.2	2.3
Oc_2Sn/TH	0.12	0.12	2.1	2.0
Ph_2Sn/TH	1.0	0.90	2.0	2.0
Cisplatin	0.80	0.57	2.9	2.1

For the organotin polymers the values are generally greater than one with the Et_2Sn and Bu_2Sn products being the largest, but small for the brain cancer cell lines. For the metallocene polymers there is again an order with respect to the particular metallocene with the zirconocene being the largest followed by the hafnocene and the smallest the titanocene. The zirconocene values are generally greater than the organotin values. For the brain cancer cell lines, only the Bu_2Sn shows values of one and higher while for the metallocenes the zirconocene shows CI_{50} values greater than one.

CONCLUSIONS

First, unless you synthesize and study the various compounds derived from different reactants there is no way of knowing which of the compounds have the greatest ability to inhibit the cancer cell lines without actually conducting the cancer-related studies. In the present study, the zirconocene compounds showed the greatest ability to inhibit all of the tested cell lines. The original use of metal-containing materials was carried out using exclusively titanocene compounds. They exhibited good ability to inhibit certain cell lines. At that time, the researchers did not know a great deal about other metal-containing compounds to study nor did they know about how to incorporate these organometallic compounds into polymers. We now know how to do this and find that while the titanocene compounds do inhibit all of the cancer cell lines we have studied, they often show greatly less ability to inhibit these cancer cell lines so that further studies should focus on zirconocene containing materials as potential anticancer agents for human studies.

Titanocene-containing compounds are one of a very few metal-containing compounds, the other being platinum-containing compounds, that have undergone human trials.[10,11]

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.[12] Some phase II clinical trials were undertaken with patients with breast metastatic carcinoma[13] and advanced renal cell carcinoma.[14] Unfortunately, low activity discouraged further clinical study.

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.[15] 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.[16]

Another major problem indicated by many researchers is the low or no solubility of titanocene-containing materials. Our polymeric titanocene-containing polymers are soluble in DMSO so even the polymeric materials are soluble, and this is not a limiting factor though in truth, the titanocene-containing polymers are less soluble compared with the zirconocene and hafnocene polymers.

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