

Antiviral and Anticancer Activity of Cisplatin Derivatives of Tilorone

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Abstract Cisplatin derivatives containing tilorone and a tilorone derivative were synthesized and characterized employing IR analysis, mass spectrometry, elemental analysis, and chain length. The polymeric cisplatin derivatives prevent growth of four transformed cell lines, L929, 143, Vero, and BS-C-1 and effectively inhibit four viruses including the DNA viruses; herpes simplex-1, vaccinia, and Varicella zoster, and the RNA virus, reovirus in the micrograms/mL range. The tilorone polymers display activity against both transformed cells and DNA and RNA viruses at biologically important concentrations.

Keywords Tilorone · Platinum-containing polymers · Cisplatin · Cancer · Viruses

1 Introduction

There are increasing pressures to develop antivirals to treat both old diseases, such as smallpox and herpes, and new diseases, such as the bird flu, AIDS and SARS [1]. Viral vaccination programs are coming under increased scrutiny, including the current smallpox vaccination, with concerns

about the occurrence of complications in people with immunodeficiency disorders. Additionally, a new climate appears to be emerging in which the acceptance of the risks inherent to vaccines is very low, the most recently casualty of this being the rotavirus vaccine [2]. Antivirals also have an advantage because they are likely to be active against a new pandemic variant, unlike viral vaccines which are generally strain specific.

Antiviral agents suffer from the problem of target specificity [3]. Viruses typically use the cells own machinery to replicate the viral genome to produce new virus particles. Unfortunately, when a specific virus target is identified, the cellular processes in uninfected cells are also compromised. Polymeric drugs offer the possibility of avoiding some of these unintended occurrences [4, 5].

Polymeric drugs offer a number of possible advantages over the currently employed small molecule drugs. Polymeric drugs can act either as the drug or as a means to release the drug over a period of time. Controlled or prolonged release of the drug allows a decreased amount of the drug to be present at any one time in normally sensitive sites such as the kidneys and liver, and increases the amount of drug remaining within the target site(s). For instance, tumor-associated vasculatures are frequently hyperpermeable to plasma proteins and other macromolecules [6–10]. These leaky vasculatures and limited lymphatic drainage, typical of tumor and missing in normal tissue, result in the accumulation of macromolecules such as polymeric drugs in the interstitial space of a large variety of tumors. This effect is known as the “enhanced permeability and retention effect”, EPR. Trapped polymeric drugs can then act over a longer time as polymeric drugs themselves or in controlled release of the active drug. This continuous release of bioactive drugs is crucial for cell-cycle-specific drugs as well as for reducing drug resistance.

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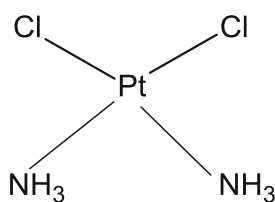
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The polymers can also act as drugs themselves, offering advantages in doing so. Healthy cells have fully functional autoimmune systems, whereas unhealthy cells, such as cancer cells, have their autoimmune systems compromised [11–15]. For instance, cancer systems that are related to p53 may be deactivated by the invading cancer, allowing polymeric drugs to combat disease effectively, by allowing the polymeric drugs to remain within the infected cells [16–22]. Conversely, the intrusion of drugs within healthy cells is resisted by healthy cell more effectively [23–25]. Other advantages have been recently reviewed [4].

Research has also shown a relationship between cancer and some viruses [26–32]. Viruses with DNA genomes commonly induce cell division in order to initiate their own replication. This is also true of some retroviruses for which an intact nuclear envelope is a barrier to integration of the viral provirus into the host genome. The manner in which these viruses induce cell division has been altered. Therefore, it is reasonable to test both the ability to inhibit cell growth and viral replication for the same compounds.

We have been surveying a number of metal-containing polymers as potential cancer and antiviral agents emphasizing organotin and polymeric derivatives of cisplatin [4, 6–25, 33–44]. Our research with platinum-containing drugs has focused on polymeric derivatives of cisplatin as anticancer drugs [4, 38–44]. Most of these polymeric drugs inhibit cancer cell growth within the same concentration range as cisplatin itself (**1**) and are less toxic than cisplatin [4]. For instance, a typical upper dosage of cisplatin (single dose) is about 4×10^{-4} g/kg for humans which is increased to about 4×10^{-3} when flushing is employed. For rats the LD₅₀ (ipr-rat) is 1.2×10^{-3} g/kg. We have injected mice on an alternate day schedule for 1 month DMSO-water (10–90% by volume) solutions containing 2×10^{-2} g/kg per dose without apparent harm using the cisplatin derivative from 1,6-hexanediamine [4].



(1) Cisplatin

Some of these cisplatin drugs have also exhibited antiviral activity [4]. The cisplatin polymer derived from tetrachloroplatinum(II) and tetramisole inhibited the EMC-D viruses that is responsible for the onset of juvenile diabetes symptoms in ICR Swiss mice [43]. In a related study,

the platinum polymer derived from methotrexate showed similar results [40].

In both of the cases cited above, of the coupling a known drug with the metal proved successful in inhibiting viral growth. It is known that a molecular complex of tilorone and RNA exhibit an antiviral effect similar to that of a polynucleotide interferon (IFN) inducers such as poly(I)-poly(C), larifan, and ridostin [4, 5]. In the current study, tilorone is coupled with platinum. Our argument for the coupling of known drugs includes attacking various diseases at multiple sites, reducing the ability of the disease to effectively resist it.

Here we describe the synthesis and results related to the ability of two tilorone-platinum polymers to inhibit various cancer cell lines and viruses.

2 Experimental

2.1 Synthesis and Physical Characterization

The reactants were employed as received. Tilorone and tilorone 11,567 were obtained from Sigma, St. Louis; and potassium tetrachloroplatinate(II) was obtained from J & J Materials, Neptune City, NJ. Synthesis is carried out in the usual manner [4]. The tilorone (5.4 mmole), dissolved in 150 mL distilled water, is added to stirred solutions of potassium tetrachloroplatinate(II) (5.4 mmole) in 15 mL distilled water at room temperature. Product precipitation begins after about an hour. The product (in greater than 90% yield) is recovered after 6 h using vacuum filtration and washed repeatedly with distilled water to remove unreacted materials. The solid is washed onto a glass petri dish and allowed to dry.

Molecular weight was determined employing a Brice-Phoenix BP-3000 Universal Light Scattering Photometer in DMSO. Refractive indices were obtained using a Bausch Lomb Abbe Model 3-L refractometer.

Solubilities were determined by placing 1–10 mg of polymer in 3 mL of liquid. The solid–liquid combinations were observed over a period of 2–4 weeks.

Infrared spectra were obtained employing KBr pellets using a Mattson Instruments galaxy Series 4020 FTIR using 32 scans and an instrumental resolution of 4 1/cm. Proton NMR were obtained using Varian-500 and Varian-400 spectrometers employing DMSO_{d6} and D₂O.

Mass spectral analysis was carried out employing two approaches. For the HR MS analysis, a direct insertion probe connected to a Kratos MS-50 mass spectrophotometer operation in the EI mode, 8 kv acceleration and 10 s/decade scan rate with a probe temperature of 350–450 °C was employed. 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 2,000–100,000. Two hundred shots were typically taken for each spectrum. Several matrix materials were employed but here only results employing 2,5-dihydroxybenzoic acid are included in the present paper.

Elemental analyses were carried out by Galbraith Laboratory, Knoxville, TN.

2.2 Biological Characterization

2.2.1 Virus and Cell Lines

The viruses used in this study were reovirus serotype 3 (ST3) strain Dearing [45, 46] propagated in mouse L929 fibroblasts (ATCC CCL-1), Vaccinia [47] virus, strain WR propagated in human 143 cells, Herpes simplex virus 1, strain GHSV-UL46 (HSV-1 ATCC VR-1544) propagated in Vero cells and Varicella Zoster virus, strain Ellen (VSV ATCC VR-137) propagated in BS-C-1 cells. Cell lines were maintained in monolayer cultures using minimal essential medium (MEM) with Earles' salts, supplemented with 5% fetal bovine serum (FBS) [48]. Cells were passaged at 1:2–1:10 dilutions according to conventional procedures using 0.05% trypsin with 0.02% EDTA.

2.2.2 Drug Preparation

The drugs were prepared by dissolving the dried material into 100% DMSO at a concentration of 10 microgram/mL. Working stocks were then generated by diluting stocks 1–10 into MEM yielding a final drug concentration of 1 microgram/mL. From these working stocks material was transferred to MEM with 5% FBS yielding the indicated final concentrations, and the media added to the cell monolayers.

2.2.3 Cytotoxicity Assays

Drug cytotoxicity was determined by plating cells at a concentration of 5×10^5 cells per well in MEM with 5% FBS using a 6-well plate and incubating the plates at 37 °C, 5% CO₂ for approximately 24 h until the cells divided to yield 1×10^6 cells per well. At this time, the media was removed and replaced with MEM with 5% FBS and the indicated drug concentration. Cytotoxicity was observed microscopically after 48 and 96 h with the addition of trypan blue to stain nonviable cells [49]. Assays were performed in duplicate.

2.2.4 Reovirus, Vaccinia, HSV-1 and VZV Plaque Reduction Assays

L929, human 143, vero or BSC-1 cells were grown to confluency in 6-well plates in MEM with 5% FBS. The media was removed and the cells infected with either reovirus, vaccinia, HSV-1 or VZV virus at serial 10-fold dilutions ranging from 1×10^6 to 10 plaque forming units (PFUs) per well in 250 microliters of MEM. After 30 min the media was removed and replaced with MEM with 5% FBS and the indicated drug concentration. After incubation at 37 °C and 5% CO₂ for 48 or 96 h the cells infected with reovirus or vaccinia virus were treated by the addition of trypan blue to stain nonviable cells. The cells infected with vaccinia virus were stained with crystal violet. The L929 cells infected with reovirus were stained with neutral red. After staining, the plaques counted [50]. The HSV-1 infected Vero cells were observed after excitation at 395 nm for fluorescent plaques [51], and the VZV infected BS-C-1 cells stained with crystal violet to observe viral plaques [52]. Assays were performed in duplicate.

3 Results and Discussion

3.1 Physical Characterization

Tilorone, 2,7-bis[2-(9-diethylamino)ethoxy]-9H-fluorene-9-one, is the first recognized synthetic small molecule that is an orally active interferon inducer. Because of its potential importance, a number of similar structures were synthesized. These derivatives are given various numbers that follow the name tilorone. Tilorone 11,567 is one of these derivatives.

The polymers are synthesized by adding equimolar aqueous solutions of potassium tetrachloroplatinate II and the tilorone with the resulting polymer captured as a precipitate. This allows a ready synthesis of the products using commercially available reactants which is advantageous for commercial application.

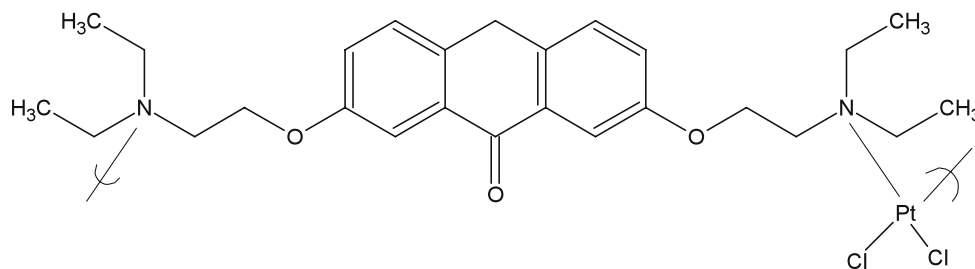
Table 1 gives the chain length data concerning the two tilorones tested in the current study. There is a marked difference in the chain lengths of the products. It is possible that the increased steric requirements about the tilorone amines is responsible for the lowered chain length in comparison to that found for tilorone 11,567.

Table 1 Tilorone polymer results

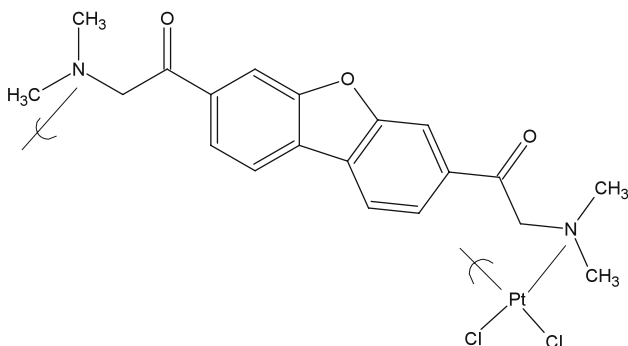
Compound	%-Yield weight	Molecular	DP
Pt-Tilorone	94	13,000	20
Pt-Tilorone 11,567	92	110,000	160

Structures of the platinum polymers are given below (2,3). The trans-effect ensures that only the cis-derivative is formed [4].

The important differences are associated with the presence of bands characteristic of the PtCl_2 and Pt–N moieties in the polymers. The bands at 324 and 286 (all bands are



(2) Product of potassium tetrachloroplatinate II and tilorone.



(3) Product of potassium tetrachloroplatinate II and tilorone 11,567.

Infrared spectra were obtained for both the monomers and polymers. The polymer spectra are similar to those of the tilorone and tilorone 11,567 as expected. Infrared band assignments are given in Table 2.

given in cm^{-1}) are assigned to the Pt–Cl stretch and correspond to literature values of 332 and 282 for cisplatin itself. [53–57] The band at about 512 corresponds to the band reported at 508 for the symmetrical Pt–N stretch and the band at about 528 corresponds to the band at 517 for the Pt–N asymmetrical stretch in cisplatin. [53–57] The presence of two Pt–N stretching bands is also significant since their presence is consistent with a cis geometry about the platinum atom [4]., [53–57] Trans geometries are found to exhibit only one Pt–N stretching band. Further, only the formation of a cis geometry from reaction of the tetrachloroplatinate with mono and diamines has been reported because of the so-called trans effect [4, 53–60] C–H associated distortion bands appear at about 1,470, 1,410, and 1,390 in the monomer and about 1,465, 1,410, and 1,390 in the polymer. C–H stretching frequencies for both aromatic and aliphatic appear about 3,055, 2,978, and 2,942 for tilorone and 3,022, 2,980, and 2,943 for the tilorone polymer. C–N stretching occurs at 1,057 and 1,013 for the tilorone itself and 1,048 and 1,012 for the tilorone polymer. Thus, infrared spectral analysis is consistent with the presence of both the tilorone or tilorone 11,567 and platinum units as well as the formation of the Pt–N bonds.

Table 2 Selected infrared bands and assignments

Assignment	Cisplatin	Tilorone	Pt–Tilorone Polymer	Pt–Tilorone 11,567 polymer
Pt–Cl St.	332, 282		324, 286	324, 286
Pt–N Sym. St	508		514	511
Pt–N Asym. St.	517		528	527
Ring carbonyl		1708	1711	1687
C–H Ar. St.		3055	3022	3043
C–H Alip. St.		2978, 2942	2980, 2943	2968, 2936
C–N St.		1057, 1013	1048, 1012	1055, 1020
C–C St.		990, 941	980, 940	990, 966
Ring St.		1604	1606	1631
CH ₂ Deformation		1465, 1412, 1390	1466, 1410, 1391	1464, 1404, 1384

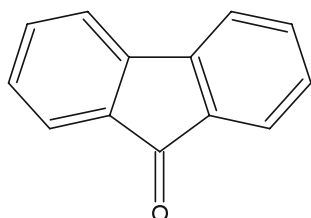
Table 3 Elemental analysis results for the platinum polyamines

Polymer Amine	Carbon (%)		Hydrogen (%)		Nitrogen (%)	
	Theory	Found	Theory	Found	Theory	Found
Tilorone	43	38	5	5	4.2	4.6
Tilorone 11,567	40	41	4	4	4.6	5.3

Table 4 Results of the HR PYR EI MS for the product derived from tilorone and tetrachloroplatinate. All ion fragments are given in Daltons

Mass	Assignment	Mass	Assignment
35	Cl	36	HCl
37	Cl	38	HCl
41	C ₂ H ₃ N	44	C ₂ H ₄ O
58	NCH ₂ CH ₂ O	72	N(C ₂ H ₅) ₂
86	N(CH ₂ CH ₂) ₂ O	100	((NC ₂ H ₅) ₂) ₂
106	Ph-C=O	126	R-C ₄ H ₄
139	R-C ₃ H ₄	410	T

where R =



and T = tilorone

The elemental analysis results are given in Table 3. It must be noted that elemental analysis for similar platinum-containing polymers is generally poor and the current values are reasonable in this light [4].

Both high resolution pyroprobe electron impact mass spectrometry, HR PY EI MS (Table 4), and high resolution electron impact matrix assisted laser desorption ionization

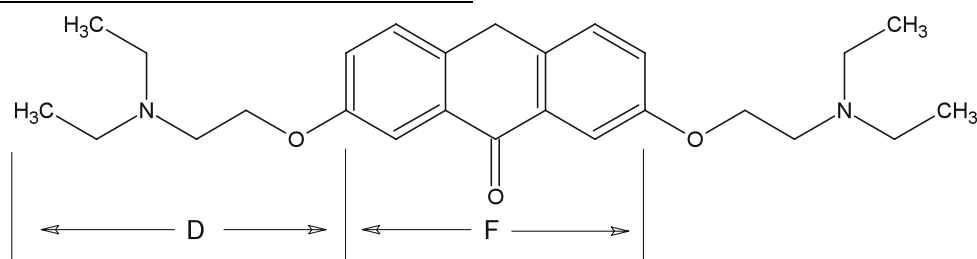
Table 5 Low mass range (100–1,500 Daltons) ion fragments for the product derived from tilorone and tetrachloroplatinate

Mass	Assignment	Mass	Assignment
100	Et ₂ NCC	116	D
179	F	280	PtN ₂ Cl
294	PtN ₂ Cl ₂	310	DFO
326	Pt(NEt ₂) ₂	342	T-NEt ₂
374	Pt(NEt ₂) ₂ Cl	382	T-Et
409	Pt(NEt ₂) ₂ Cl ₂	411	T
425	Pt(NEt ₂) ₂ Cl ₂ C	437	Pt(NEt ₂) ₂ Cl ₂ CC
452	Pt(NEt ₂) ₂ Cl ₂ CCO	479	Pt(NEt ₂) ₂ Cl ₂ CCOCC
729	U+NEt	801	U+Et ₂ NCCO
843	U+DF-Et	867	U+DF
955	U+PtNCl ₂	983	U+DF
1022	U+PtCINEt+CCO	1134	U+Pt(NEt ₂) ₂ Cl ₂ +CCO
1204	2U-2NEt ₂	1275	2U-NEt ₂
1465	2U+Et ₂ NCCO		

mass spectrometry, HR EI MALDI MS were carried out on the monomers and polymers. For brevity, only the results for tilorone and the tilorone polymers will be reported. Results for tilorone 11,567 are similar.

The isotopic abundance of Cl(35) to Cl(37) is 3:1. For the tilorone polymer the ratio of Cl(35)/Cl(37) is 3.1 and the ratio of HCl(35)/HCl(37) is 2.8 consistent with these ions being chlorides. The presence of these ions is consistent with the presence of the PtCl₂ moiety. The ion fragments from 43 to 100 are derived from the diethylaminoethoxy chain of tilorone. The ion fragment at 410 is derived from tilorone.

MALDI MS results are given in Table 5, which shows the low mass range ion fragments for the tilorone polymer. A number of abbreviations are employed to convey assigned structures. These are U = one unit, 2U = two units, etc.; D = diethylaminoethoxy arm designated as D below (4); F = fluorenone ring designated as F (4), T = tilorone, and C = CH₂.

**(4)**

For comparison, Table 6 contains the major ion fragments created from the MALDI MS of tilorone itself. There exists a number of similarities in the lower mass range between the tilorone polymer and tilorone itself as expected with the ion fragments at 116, 294, 312, and 411 the same in both spectra.

Table 7 contains isotopic matches for ion fragments that contain both platinum and chlorine, each containing significant contributions to the resulting isotopic abundances given in the extreme two left columns. The agreements are consistent with the presence of the PtCl_2 moiety in these ion fragment clusters.

Table 8 contains high mass ion fragments for the tilorone polymers. Assignments include ion fragments to over 20 units, which are consistent with the results from light scattering photometry that the product is a low molecular weight polymer. As in other cases when using MALDI MS loss of fragments occurs only at the site(s) of bond scission [35, 37].

The results are consistent with the product being a high molecular weight polymer in agreement with the results found for light scattering photometry.

Table 6 Low mass range (100–2,000 Daltons) ion fragments for tilorone

Mass	Assignment	Mass	Assignment
116	D	212	F+2O
234	DF–2Et	252	F+2O, CCN
296	DF	312	DFO
411	T	435	T, Na

Table 7 Isotopic abundances for selected ion fragments containing PtCl_2 (>10%) derived from the tilorone polymer

PtCl_2		$\text{Pt}(\text{NEt}_2)_2\text{Cl}_2$		$\text{Pt}(\text{NEt}_2)_2\text{Cl}_2\text{C}$	
Mass	Calculated % intensity	Mass	% intensity	Mass	% intensity
264	72	407	79	423	70
265	73	408	75	424	72
266	100	409	100	425	100
267	47	410	50	426	47
268	57	411	60	427	60
270	15	413	15	429	10

Table 8 High mass range (10,000–50,000 Daltons) ion fragments for the product derived tilorone and tetrachloroplatinate

Mass	Assignment	Mass	Assignment
10644	16U–D, 2Cl	11252	17U– PtCl_2
12462	18U+DF	14154	21U–Cl

The two employed MS approaches complement one another. The PYR MS detects lower mass ion fragments allowing the identification of the PtCl_2 moiety through the presence of chlorine atoms. MALDI MS allows the identification of higher mass ion fragments as well as detection of the presence of platinum through isotopic abundance matches.

4 Biological Characterization

4.1 Cytotoxicity

Each cell line is specially chosen to be compatible to support growth for the particular virus. The cell lines are all transformed cell lines with cancer-like characteristics and employed to measure drug effectiveness as potential anti-cancer agents. BSC-1 cells are African green monkey kidney epithelial cells; Mouse L929 are fibroblast cells; vero cells are African green monkey kidney epithelial cells; and human 143 cells are fibroblast bone osteosarcoma cells.

The toxicity of the tested compounds towards the cell lines employed in this study is given in Table 9. The cell line and viral replication results are presented as means of four experiments using duplicate samples in each experiment.

The tested compounds, Table 9, showed similar toxicity to each of the cell lines. The cells are able to tolerate relatively high levels of the tetrachloroplatinate(II), but good inhibition of the tested cell lines occurs for the tilorone polymers.

4.1.1 GI_{50} Values

The potential for these polymers to act as chemotherapeutics was examined by determining the concentration of material necessary to inhibit growth by 50%, GI_{50} . Along

Table 9 Minimum cell cytotoxicity concentration of the compounds

Compound	MCC ($\mu\text{g}/\text{mL}$)			
	L929	143	Vero	BS-C-1
K_2PtCl_4	100 \pm 9.0	75 \pm 6.0	100 \pm 9.0	75 \pm 6.0
Pt–Tilorone 11,567	200 \pm 21.0	150 \pm 12.0	150 \pm 12.0	150 \pm 14.0
Pt–Tilorone	125 \pm 12.0	100 \pm 9.0	150 \pm 12.0	100 \pm 9.0
Acyclovir	500 \pm 42.0	425 \pm 32.0	400 \pm 42.0	440 \pm 39.0
Cisplatin	25 \pm 2.0	25 \pm 2.0	25 \pm 2.0	25 \pm 2.0

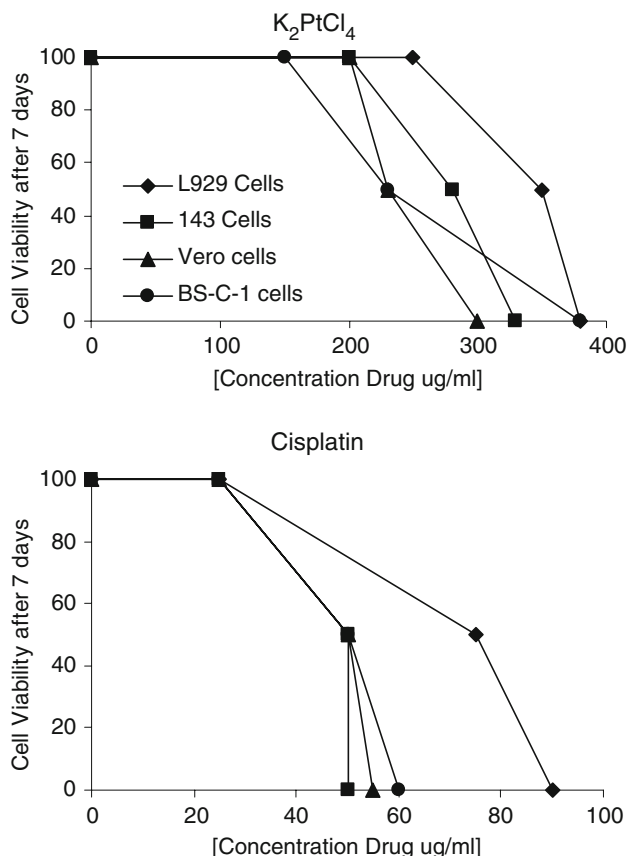
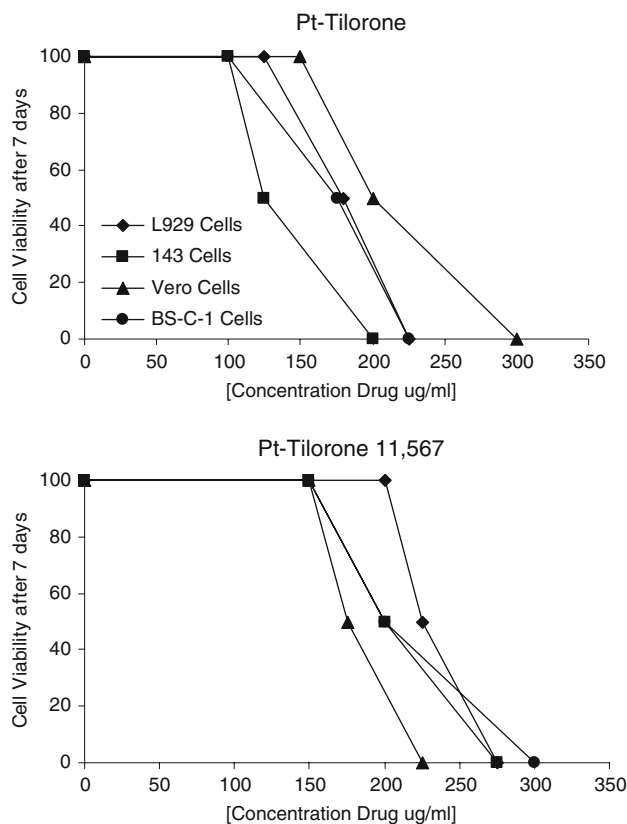
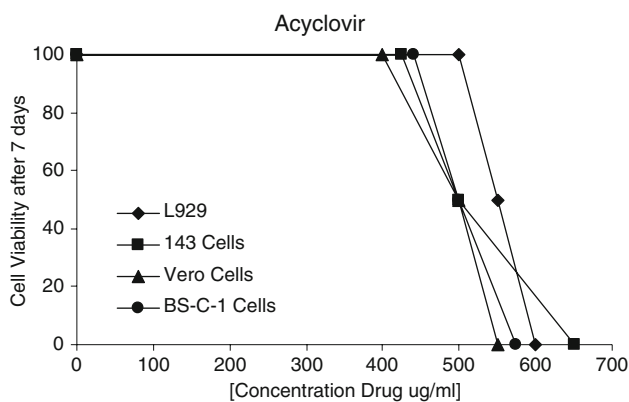
MCC = Minimum cytotoxicity concentration that causes a reduction in cell viability >10% as measured by trypan blue dye exclusion after 7 days (\pm) one standard deviation of the mean

Table 10 Chemotherapeutic activity of the compounds (GI₅₀)

Compound	GI ₅₀ (μg/mL)			
	L929	143	Vero	BS-C-1
K ₂ PtCl ₄	200 ± 20.0	175 ± 12.0	200 ± 16.0	175 ± 20.0
Pt-Tilorone 11,567	300 ± 22.0	350 ± 30.0	350 ± 29.0	350 ± 30.0
Pt-Tilorone	325 ± 30.0	300 ± 28.0	350 ± 32.0	300 ± 30.0
Acyclovir	700 ± 55.0	625 ± 52.0	650 ± 51.0	640 ± 52.0
Cisplatin	75 ± 6.0	50 ± 4.0	50 ± 4.0	50 ± 3.0

GI₅₀ = (Growth Inhibition 50%) Drug concentration that results in a 50% reduction in cell growth after 7 days (±) one standard deviation of the mean

with the two tested polymers, the results are also given (Table 10) for cisplatin, the most widely used chemotherapy drug; acyclovir, the most widely employed antiviral agent; and K₂PtCl₄. The toxicity curves used to determine the GI₅₀ values are shown in Figs. 1–3 for each of the five tested compounds in each of the four cell lines. Cisplatin shows a GI₅₀ of about 50 μg/mL (Fig. 1). Thus, the polymers have GI₅₀ values only six to seven times that found for cisplatin. For comparison with some of the other

**Fig. 1** Cell inhibition for cisplatin and tetrachloroplatinate (II) as a function of drug concentration**Fig. 2** Cell inhibition as a function of polymer concentration**Fig. 3** Cell inhibition as a function of acyclovir concentration

polymeric cisplatin drugs made by us, the polymers derived from 1,6-hexanediamine and 1,4-phenylenediamine show GI₅₀ values of about 40 μg/mL against L929 cells [41]. The products from 4-nitrohydrazine and phenylhydrazine show GI₅₀ values at 8 μg/mL against L929 cells [61]. Thus, while the tilorone polymers exhibit cell inhibition within the same general concentration range as cisplatin, it will be seen that their real potential application is as antiviral agents.

The viruses were chosen to represent a broad range of virus. The reovirus ST3 virus is a RNA virus that is currently being investigated because of its ability to inhibit certain cancer cells while leaving normal cells alone. Generally, drugs that are capable of inhibiting one RNA virus will be effective against other RNA viruses. This particular virus is responsible for many respiratory and enteric infections. The other viruses are all DNA viruses and the activity against different DNA viruses must be studied individually. Vaccinia is the vaccine strain for smallpox; herpes simplex is responsible for at least 45 million infections in the US yearly, or one out of five adolescents and adults; and varicella zoster is responsible for chickenpox and shingles.

Table 11 gives the results of preliminary studies involving the capacity of the various test compounds to inhibit the viruses. The results are presented as the minimum inhibitory concentration of the compound (MIC) needed to reduce virus plaque formation by 50%.

Potassium tetrachloroplatinate II showed little inhibition of the tested viruses. Both of the tilorone polymers exhibited good inhibition of all of the tested viruses at low concentrations. For comparison, the organotin polymers of norfloxacin, ampicillin and acyclovir showed good inhibition of these same viruses but at much higher concentrations, within the range of 2 $\mu\text{g}/\text{mL}$ [33, 34], while the tilorone polymers showed good activity at 0.1–0.2 $\mu\text{g}/\text{mL}$, well below that of the organotin polymers.

The analogous cisplatin polymer derived from methotrexate showed good activity against HSV-1 (about 4 $\mu\text{g}/\text{mL}$) and VZV (about 6 $\mu\text{g}/\text{mL}$), DNA viruses whose genome replication and mRNA transcription occur in the nucleus using viral DNA polymerases and RNA transcription using the cellular RNA polymerase [4]. Some activity is seen against vaccinia virus, a DNA virus with cytoplasmic DNA replication utilizing viral DNA polymerase and uniquely cytoplasmic transcription using a viral RNA polymerase. No activity is seen against reovirus, a dsRNA virus with cytoplasmic replication utilizing a viral

RNA polymerase. The target of the drug that appears to have the greatest impact against these viruses, is the cellular RNA polymerase located in the nucleus associated with the production of viral and cellular mRNA. Activity against vaccinia and reovirus is greatly reduced compared against HSV-1 and VZV and both vaccinia and reovirus utilize viral polymerases to produce their mRNA transcripts. Again, the concentration levels to achieve good inhibition of HAV-1 and VZV are much higher than that found for the tilorone polymers.

We have already noted that there is a relationship between some cancers and viruses [26–32]. Viruses use and generally modify cellular machinery to replicate the viral genome, produce viral proteins and assemble new virus particles. Many cancers also modify the cellular machinery to replicate. A number of these modifications to cellular machinery are common to both viral infections and tumor cell growth. A recent report by Reeves and others [62] found the antitumor drug Gleevec is capable of inhibiting vaccinia virus and raises the possibility that such drugs may be given at the same time as the vaccinia vaccination to prevent vaccine complications. These antitumor/antiviral drugs are a new class of cellular targets whose disruption will lead to a halt in cell replication in virus-infected cells, and a block in virus replication. This class of compounds interacts with cellular targets utilized both by the process of transformation and virus infection leading to cell replication.

Tilorone antiviral activity is most likely due to a direct inhibition of viral DNA synthesis for both HSV-1 and VZV-infected cells [62–65]. Tilorone is mostly used as an antiviral agent as its hydrochloride. It is the first recognized synthetic, low-molecular-weight compound that is an orally active interferon inducer, and is also reported to have anti-neoplastic and anti-inflammatory actions [44, 66–69]. Tilorone hydrochloride has been shown to inhibit HSV-1 and vaccinia at concentrations of 10 $\mu\text{g}/\text{mL}$ [70, 71]. The researchers noted that the adsorption of the virus was not affected by the drug, and the penetration of the deoxyribonucleic acid of the input virus into the cytoplasm and nuclei proceeded normally when tilorone hydrochloride was present. However, newly synthesized viral deoxyribonucleic acid was not detectable under these conditions and there was a remarkable decrease in the rate of viral polypeptide synthesis. Further, virus particles were not formed. They noted that the inhibition of herpes virus growth by tilorone hydrochloride was dependent on the presence of the drug in the cultures. Pretreatment of the cells with the drug did not result in resistance to herpes virus infection after the removal of the drug. The researchers tested a number of ssRNA viruses and no antiviral activity was measured. By comparison, we do see activity against a dsRNA virus, reovirus. This is not

Table 11 Plaque reduction assay

Compound	MIC ($\mu\text{g}/\text{mL}$)			
	Reovirus ST3	Vaccinia WR	HSV-1	VZV
K_2PtCl_4	>100	>75	>100	>75
Pt–Tilorone 11,567	20.5 \pm 2.0	16.5 \pm 2.0	10.5 \pm 2.0	9.2 \pm 1.2
Pt–Tilorone	11.5 \pm 1.4	8.3 \pm 1.1	8.5 \pm .079	8.8 \pm 1.0
Acyclovir	>250	>200	.076 \pm 0.1	2.0 \pm 0.7
Cisplatin	>25	>25	>25	>25

MIC = Minimum inhibitory concentration required to reduce virus plaque number by 50% (\pm) one standard deviation of the mean

unexpected because reovirus, like a number of RNA viruses, requires cellular DNA synthesis to take place prior and/or during viral replication [72]. Tilorone, by inhibiting cellular DNA synthesis, can and does inhibit the replication of a number of DNA and RNA viruses.

For added comparison, the results for acyclovir, a commercial antiviral often used to inhibit the herpes viruses tested, are included. Inhibition of HSV-1 by acyclovir requires a concentration level of about 0.08 $\mu\text{g}/\text{mL}$ and 2 $\mu\text{g}/\text{mL}$ for VZV. This compares to a concentration about 10 $\mu\text{g}/\text{mL}$ for the tilorone-Pt polymers against both of these viruses. But, in comparing the MIC values for acyclovir for Reovirus ST3 (>250 $\mu\text{g}/\text{mL}$) and Vaccinia WR (>200 $\mu\text{g}/\text{mL}$) the two tilorone polymers have much lower MIC values (10–20 $\mu\text{g}/\text{mL}$ for Reovirus ST3 and 8–17 $\mu\text{g}/\text{mL}$ for Vaccinia WR). Thus, the two tilorone polymers are broader acting antiviral agents in comparison to acyclovir and show promise as additional antiviral agents in the treatment against viral infections.

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