Cisplatin Derivatives as Antiviral Agents

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1 Introduction

The spotlight is again on the urgent need for the development of antivirals to treat both old and emerging virus outbreaks. The recent attempt to reinitiate the smallpox vaccination program to deal with biological terrorism has been halted, in part resulting from observation of adverse cardiac events following vaccination, and has resulted in the Centers for Disease Control recommending that people at risk for heart disease should be excluded from the vaccination program. Although the number and severity of adverse reactions associated with most if not all vaccines does not appear to be increasing, the percentage of the human population susceptible to these problems does appear to be increasing. Increased longevity and both infectious and environmental factors are producing a population increasingly harboring immunocompromised individuals for whom vaccinations are unworkable.

Diseases caused by old foes such as influenza always maintain the potential to initiate new pandemics of disease. Antivirals, unlike vaccines have the potential to act against new variants of old viruses and new viruses such as SARS [1,2], and the bird flu, and as interventions in biological terrorism. 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 [3].

Antivirals, however, all suffer from the problem of target specificity [4,5. Viruses, for the most part, utilize cellular machinery to replicate the viral genome and produce new virus particles. In an attempt to target viral replication, the cellular processes in uninfected cells are also undesirably affected. Polymeric drugs offer the opportunity to avoid some of these effects. This is reviewed in recent articles and briefly described following [6,7].

We have been surveying a number of metal-containing polymers as potential antiviral agents emphasizing both platinum and organotin-containing polymers [8–11]. Polymeric drugs offer many potential advantages over monomeric or small molecule drugs. These reasons have been described elsewhere and will be briefly described later in this chapter.

Researchers have suggested that at least some cancers have a viral relationship. Thus, we have begun testing polymers that show good anticancer activity against a variety of viruses.

Recently, we first tested a number of organotin products derived from known antibacterial drugs, namely ciprofloxacin, ampicillin (Scheme 8.1), and norfloxacin (Scheme 8.2). All of these products exhibited some antiviral activity against a number of viruses, that is reovirus ST3, vaccinia virus, herpes simplex virus (HSV-1), and varicella zoster virus (VZV) [8,9]. The organotin polymers from norfloxacin and ampicillin showed total inhibition to virus growth at concentrations of about 2 mg/ml, whereas norfloxacin and ampicillin, themselves, exhibited no viral inhibition. We also looked at organotin products derived from the known antiviral drug acyclovir [9]. Again, the organotin polymers showed
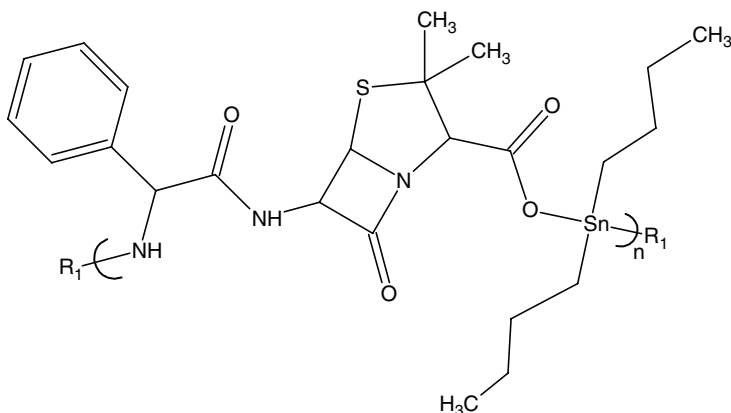
Scheme 8.1 Dibutyltin-ampicillin polymer
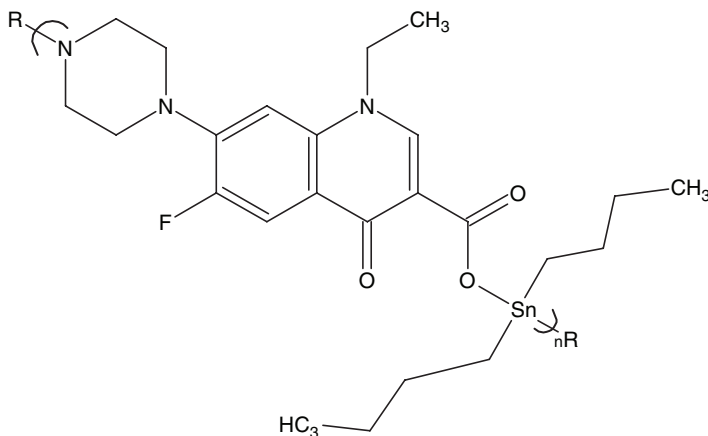
Scheme 8.2 Dibutyltin-norfloxacin polymer

inhibition of the viruses at lower concentration that found for acyclovir itself. Thus, the combination of known drugs with organotin moieties within polymers appear to be effective antiviral agents, more effective than either of the reactants themselves.

Our interest in these organotin-containing polymers is also derived from the fact that they are potent anticancer agents capable of inhibiting cancer cell growth at concentrations within the same range and less than that of cisplatin itself [12–19]. Further, these organotin polymers are much less toxic than cisplatin, the most widely used anticancer drug.

We have been investigating a number of polymeric derivatives of cisplatin as anticancer drugs for about 30 years [20]. Again, we have synthesized polymeric drugs that inhibit cancer cell growth within the same concentration range as cisplatin itself and these drugs, are again, much less toxic. We have also been investigating some of these polymeric cisplatin derivatives as antiviral agents. The use of polymeric derivatives of cisplatin as antiviral agents is the focus of this chapter.

2   Inhibition

The majority of known viruses are RNA viruses. It is not surprising that they also cause the majority of human diseases. The following are some of the more familiar diseases caused by RNA viruses:

- common cold
- poliomyelitis
- hepatitis
- encephalitis
- yellow fever

- rubella
- influenza
- measles
- mumps
- various hemorrhagic fevers

2.1 Features of an Ideal Antiviral Drug

Features of an ideal antiviral drug might include the following: effective inhibition of an essential viral process, mechanism to prevent drug-resistant viruses from developing, broad-spectrum activity against RNA and DNA viruses, and no negative effect on host cell processes.

2.2 Strategies for Antiviral Therapy

Most antivirals target one of five major viral processes: (1) attachment of the virus to the host cell, (2) penetration and/or uncoating of the virus to release the viral nucleic acid into the host cell, (3) replication of the viral genome, (4) viral gene expression to produce viral proteins, and (5) assembly and maturation of the virus structure and the release of the progeny virions with or without lysis of the host cell.

2.3 Attachment

Virus attachment can be inhibited by two broad methods which are described below.

1. Agents that mimic the viral attachment protein (VAP) can be introduced into the infected host. These VAPs will then bind to the cellular receptor and block the virus binding [21]. Anti-idiotypic antibodies can be produced that mimic the VAP. When these antibodies are introduced into the host they bind the cellular receptors that would normally be available to infectious virus. This effectively "blocks" the viral receptor on the cells and prevents the virus from attaching and infecting the cell [22]. Natural ligands of the viral receptor can be employed that bind the receptor and block virus use of this receptor. One example of this is vaccinia virus and the epidermal growth factor (EGF) receptor [23]. The fourth possibility is the use of synthetic ligands that resemble the receptor-binding domain of the VAP itself. These peptides will bind a cell receptor and render the receptor unable to bind the VAP, preventing virus infection [24,25].
2. Agents that mimic the viral receptor on the host cell and function by binding the VAP. Antibodies to the VAP, naturally produced as the humoral response to most

viruses, bind the VAP and prevent its interaction with host cell receptors [26,27]. Anti-idiotypic antibodies can be produced that mimic the cell receptor or extraneous receptors such as rsCD4 used by HIV [28]. When introduced into the host these antibodies serve as "binding targets" for the virus, but unlike the cells normally expressing these receptors, cannot be infected or support virus replication [28,29]. Additionally, synthetic receptor mimics can be produced to bind virus before it has an opportunity to bind cell receptors. One example of this approach is the use of sialic acid derivatives to bind influenza virus [30,31].

2.4 Penetration and Uncoating

These processes have proven difficult to examine at the molecular level for most viruses and therefore it has been difficult to specifically target these stages of the virus life cycle. Uncoating is for the most part mediated by cellular enzymes but like penetration, is often influenced by one or more virus proteins [32,33].

Pleconaril is a broad spectrum anti-picorna virus agent [34]. It is a small cyclic drug which binds to a canyon pore of the virus. In doing so it blocks the attachment and uncoating of the viral particle [35,36].
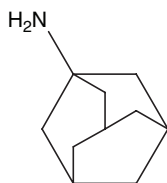
Amantadine (Scheme 8.3), and rimantadine (Scheme 8.4) are active against influenza A viruses. The action of these closely related agents is complex and incompletely understood, but they are believed to block cellular membrane ion channels [37,38]. Both drugs target the influenza A matrix protein (M2). Drug-treated cells are unable to lower the pH of the endosomal compartment (a function normally controlled by the M2 gene product), a process which is essential to induce conformational changes in the HA protein to permit membrane fusion.

2.5 Genome Replication

Many viruses have evolved their own specific enzymatic mechanisms to divert cellular energy to the replication of viral genomes. Very often there are sufficient differences between viral and cellular polymerases to provide a target for an antiviral agent without harming the unaffected agents. This strategy has yielded the majority of the antiviral drugs currently in use. Most of these drugs function as polymerase substrate, as nucleoside/nucleotide analogues. The toxicity of these drugs varies considerably from some which are well tolerated such as acyclovir [39] to others which are highly toxic such as IdU/TFT [40] and AZT [41,42]. There is a serious problem with the pharmacokinetics of these nucleoside analogs, as most typically have short serum half lives of 1 to 4 hours [43]. Nucleoside analogues are in fact pro-drugs, meaning that to be activated they need to be phosphorylated. Acyclovir [44] (Scheme 8.5), is phosphorylated by the herpes simplex virus (HSV) thymidine kinase 200 times more efficiently than by cellular enzymes. Gancyclovir [45] (Scheme 8.5) is 10 times more
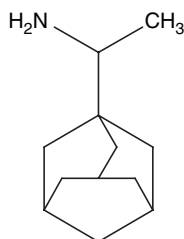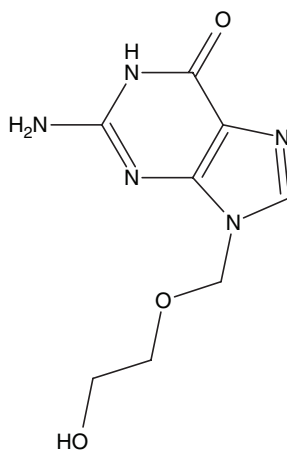
3. Amantadine

HCl

Scheme 8.3 Amantadine

4 Rimantadine

HCl

Scheme 8.4 Rimantadine

5 Acyclovir

Scheme 8.5 Acyclovir

effective against cytomegalovirus (CMV) than acyclovir since it is specifically phosphorylated by a CMV-encoded kinase not present in HSV.

Other nucleoside analogs active against herpesviruses have been derived from Acyclovir and Gancyclovir and include **Schemes 8.6–8.14**. Additional nucleoside analogues with activity against HIV are shown in Schemes 8.15–8.17.

2.6 Gene Expression

Most viruses are heavily dependent upon the cellular machinery for transcription of viral genomes, mRNA splicing, translation, and protein transport. Unlike genome replication, uniquely viral proteins are not involved in these processes and to date none have been utilized as targets for antiviral therapy.

2.7 Assembly, Maturation, and Release of Progeny Virus

For the majority of viruses, assembly, maturation, and release of progeny virus processes are poorly understood. Two drugs with anti-influenza activity are available. These are Relenza [69,70] taken as an aerosol and Tamiflu [71,72] taken as a pill. Tamiflu is active against both influenza A and B strains. Both of these drugs function as neuraminidase inhibitors and prevent the release of budded viruses from the cell.

6 Gancyclovir
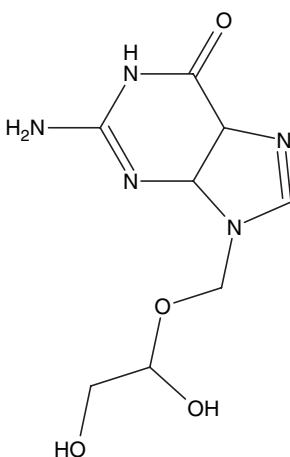
Scheme 8.6 Gancyclovir
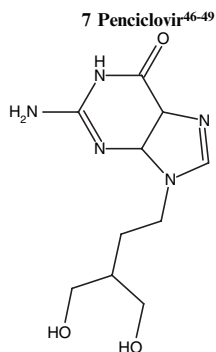
7 Penciclovir[46-49]

Scheme 8.7 Penciclovir [46–49]
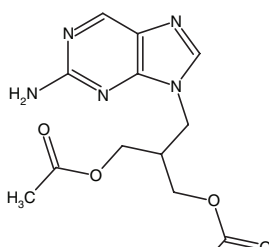
Scheme 8.8 Famciclovir [50,51]
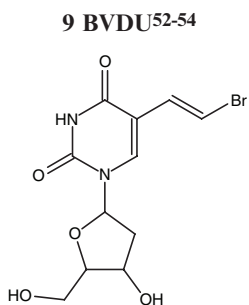
9 BVDU[52-54]

Scheme 8.9 BVDU [52–54]

2.8 Additional Antiviral Drugs

Foscarnet [73,74](Scheme 8.18) is a first line treatment for CMV retinitis and a treatment for CMV colitis if ganciclovir therapy is ineffective or not tolerated. Foscarnet crosses the blood–brain barrier and can treat susceptible infection in the

10 Brovavir[55]
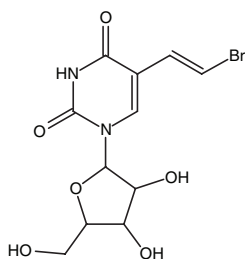
Scheme 8.10 Brovavir [55]
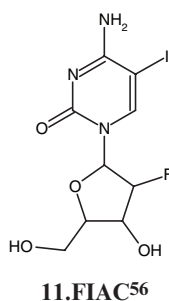
11.FIAC[56]

Scheme 8.11 FIAC [56]
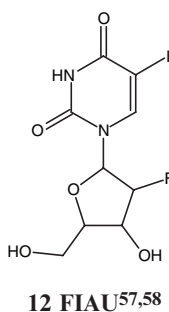
12 FIAU[57,58]

Scheme 8.12 FIAU [57,58]

brain. Strains of herpes that are resistant to treatment with acyclovir can be treated with foscarnet. Idoxuridine (IdU) [52] (Scheme 8.19) acts by irreversibly replacing thymidine in newly synthesized DNA and producing an abnormal and essentially nonfunctional DNA molecule. The drug acts on viral and host cell DNA and is highly toxic to host cells. Because of its high systemic toxicity, IdU has been
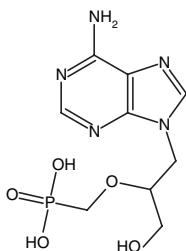
13 (S)-HPMPA [59]

Scheme 8.13 (*S*)-HPMPA [59]
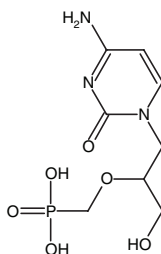
14 (S)-HPMPC[60-63]

Scheme 8.14 (*S*)-HPMPC [60–63]
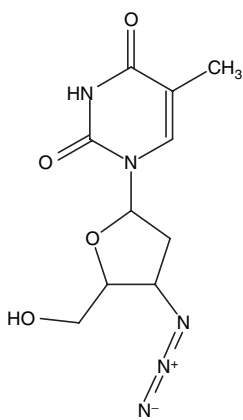
15 AZT[64,65]

Scheme 8.15 AZT [64,65]
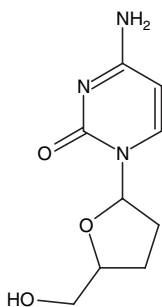
16 ddC[66,67]

Scheme 8.16 ddC [66,67]
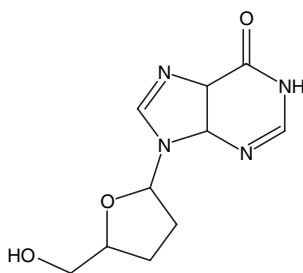
17 ddI[68]

Scheme 8.17 ddI [68]

18 Foscarnet
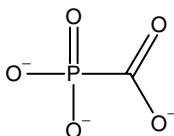
Scheme 8.18 Foscarnet

limited to topical therapy of herpes simplex keratoconjunctivitis. Ribavirin is a guanosine analog that inhibits the replication of many RNA and DNA viruses. Ribavirin (Scheme 8.20) is thought to inhibit messenger RNA formation. Ribavirin exhibits inhibitory activity in vitro against respiratory syncytial virus (RSV), influenza A and B, HSV-1, HSV-2, and many other viruses. Vidarabine (adenine arabinoside, ara-A) (Scheme 8.21) interferes with viral DNA synthesis and is effective in the treatment of HSV infections.

19 IdU
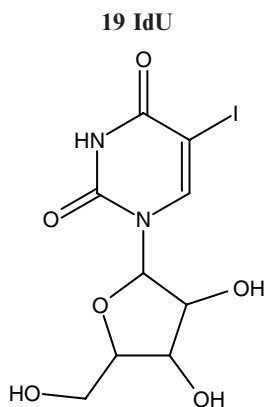
Scheme 8.19 IdU
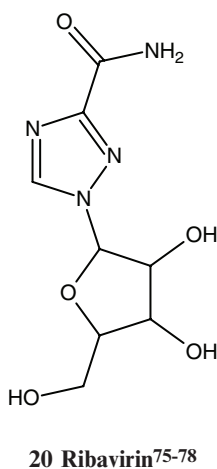
20 Ribavirin[75-78]

Scheme 8.20 Ribavirin [75–78]

3 Currently Approved Platinum-Containing Drugs

Although thousands of cisplatin analogues have been synthesized and screened only about 28 platinum compounds have entered clinical trials as anticancer agents [6,9]. Of these only four are currently approved. Those approved are cisplatin (Scheme 8.22), carboplatin (Scheme 8.23), oxaliplatin (Scheme 8.24), and nedaplatin (Scheme 8.25). Only the first two are commercially available for general use in the treatment of cancer.

Next to cisplatin (Scheme 8.22), carboplatin (Scheme 8.23) is the most widely used metal-containing anticancer drug. Although it is similar to cisplatin in its cell-killing ability, it shows moderate effectiveness with some malignancies that
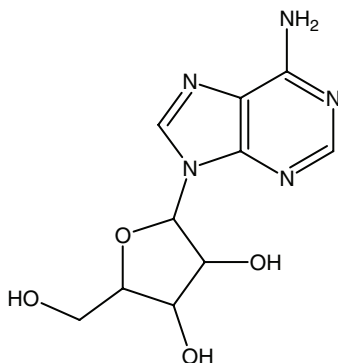
21 Vidarabine[79,80]

Scheme 8.21 Vidarabine [79,80]
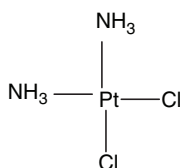
22 Cisplatin, Cis-DDP
(Cis-diaminedichloroplatinum(II)),

Scheme 8.22 Cisplatin, *Cis*-DDP (*Cis*-diaminedichloroplatinum(II))
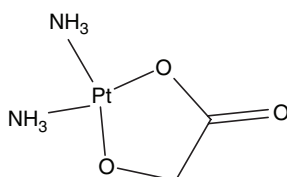
24 Nedaplatin
(Cis-diamineglycolatoplatinum (II))

Scheme 8.23 Carboplatin (Diamine cyclobutane dicarboxylate platinum(II)); *cis*-diamine-1,1-cy clobutanedicarboxylatoplatinum(II);*cis*-diamine-(cyclobutane-1,1-dicarboxylato)-platinum(II)

are less responsive to cisplatin such as non-small cell lung cancer. It also offers a different pharmacokinetic behavior. The presence of the bidentate carboxylate moiety gives decreased rates of reaction with the biological environment. Thus, it shows less nephrotoxicity and is preferred for patients suffering from kidney failure. It also shows a reduced rate of serum protein binding with only 10 to 20%
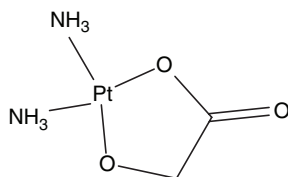
24 Nedaplatin
(Cis-diamineglycolatoplatinum (II))

Scheme 8.24 Nedaplatin (*cis*-diamineglycolatoplatinum (II))
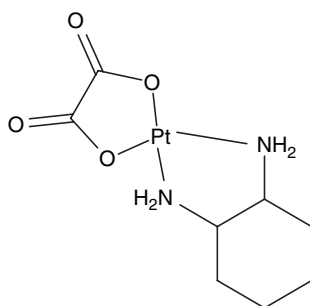
25 Oxaliplatin
(Oxalatoplatinum(II))

Scheme 8.25 Oxaliplatin (Oxalatoplatinum(II))

irreversibly bound to protein. This results in greater bioavailability and larger concentrations of the administered drug to about a five fold extent.

There are a number of good reviews covering carboplatin and related drugs [81–84].

4   Active Form of Cisplatin

While the active form of polymeric derivatives of cisplatin may be different, it may be informative to know how the active form of cisplatin itself as a beginning point in understanding how polymeric forms are active against both cancers and viruses.

In aqueous solution cisplatin is known to undergo spontaneous hydrolysis. The reaction produces species such as monoaquo platinum and diaqua platinum complexes, as shown in Eq. 8.1, arising from nucleophilic substitution in water.

$$
\begin{array}{ccccc}
\underset{H_3N}{H_3N}\diagup\!\!\!\diagdown\underset{Cl}{Cl} & & \underset{H_3N}{H_3N}\diagup\!\!\!\diagdown\underset{OH_2^{+1}}{Cl} & & \underset{H_3N}{H_3N}\diagup\!\!\!\diagdown\underset{OH_2^{+1}}{OH_2^{+1}} \\
\end{array}
$$

H₃N Cl   H₃N Cl   H₃N OH₂⁺¹
\ /   \ /   \ /
 Pt + H₂O —> Pt + H₂O —>   Pt   (8.1)
/ \   / \   / \
H₃N Cl   H₃N OH₂⁺¹   H₃N OH₂⁺¹
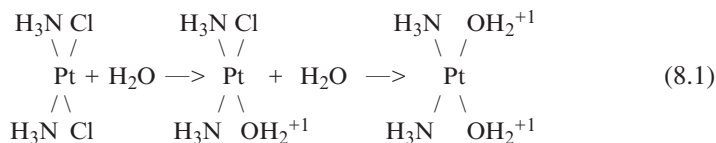
Additionally, other aqueous species can exist including hydroxy complexes such as Schemes 26 and 27. The actual form of the hydroxyl species is pH dependent. At a pH of 7.4, 85% of the monohydrated complex will exist in the less reactive dihydroxy form. Lowering the pH to 6.0 results in the most common form (80%) being the monohydrate species. Thus, the number of possible aquated forms derived from cisplatin is high and dependent on pH, temperature, time, and the concentration of associated reactants such as the chloride ion and ammonia. Figure 8.1 contains structures of some of these aquated forms of cisplatin including ones already noted.

The relatively high chloride concentration (about 100 mM) in blood minimizes hydrolysis and the formation of aquated species [9,13,85,86]. Once inside of the cell, where the chloride ion concentration is much lower (~4 mM), hydrolysis readily occurs giving a number of aquated species including the diaqua complex. At 37 °C the half-life for the completion of the formation of the diaqua complex is 1.7 hours with an activation energy of about 20 Kcal/mol (80 KJ/mol) [9,13,85,86].

Although the active form within the cell is believed to be the monohydrated structure (Scheme 8.28), the "preferred" extracellular species contains two *cis*-oriented leaving groups that are normally chloride ligands. As noted before,

H₃N   OH₂⁺¹
\ /
Pt
/ \
H₃N   OH
26

Scheme 8.26

H₃N   OH
\ /
Pt
/ \
H₃N   OH
27

Scheme 8.27
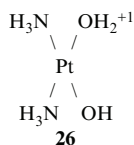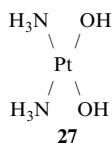
H₃N   OH₂⁺¹   H₃N   OH
  \ /   \ /
   Pt   Pt
  / \   / \
H3N OH   H₃N   OH
  26   **27**
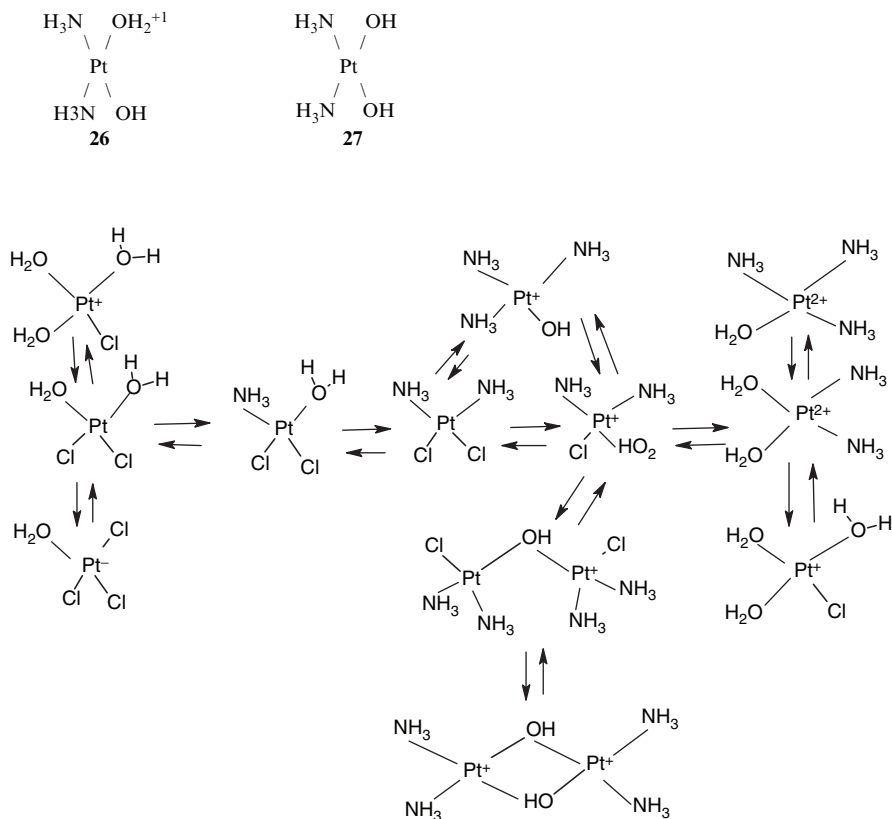
Figure 8.1 Selected aquated forms of cisplatin

H₃N   OH₂⁺
  \   /
   Pt
  /   \
H₃N   OH
    28
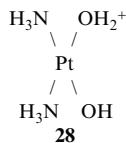
Scheme 8.28

due to the high chloride ion concentration in blood these leaving groups will remain in position resulting in the molecule being electrically neutral until it enters the cell.

As noted above, *cis*-DDP enters cells by diffusion where it is converted to an active form. This results from the lower intracellular chloride concentration

which promotes ligand exchange of chloride for water thus formation of the active aquated complex. Thus, the platinum-containing complex should be neutral to enter the cell and labile chloride groups need to be present to form the active species within the cell [87].

The antineoplastic activity of cis-DDP appears to be related to its interaction with DNA nucleotides as a monoaquo species [15]. The monohydrated complex reacts with the DNA nucleotides forming intra and interstrand crosslinks. Of the four nucleic acid bases, *cis*-DDP has been shown to preferentially associate with guanine. The most common are intrastrand crosslinks between adjacent guanines [6].

There are several possible crosslinks with DNA. One favored interstrand option occurs between the 6-NH groups of adenines on opposing strands in an A-T rich region [13,83–86,88–99]. This is because these groups are approximately 3.5 Å apart, close to the 3 Å distance between the *cis* leaving groups on the platinum. The second favored option is cross-linking occurring between the amino groups of guanine and cytosine in opposing strands. This is favored because the platinum is at right angles to the bases that in turn are coplaner with one another. This implies that the bases will have to either "bend down" or "turn edge" to achieve the necessary configuration to bind to the platinum complex. This binding pattern is believed to lead to perturbation of the secondary structure and minor disruption of the double helix. This is sufficient to cause inhibition of DNA replication and transcription with eventual cell death, yet too small to cause a response by damage recognition proteins and consequent excision of the affected segment and repair of the strand [13,83–86,88–103].

Although there are a variety of types of crosslinks that can be formed by cisplatin, the intrastrand crosslinks are the most common. Most of these crosslinks can be repaired but at least one type of interstrand crosslink may not induce response of cellular repair enzymes. Further, whereas intrastrand crosslinks are important in describing the activity of cisplatin, inducing apoptosis also appears to be a factor in the mechanism of cisplatin's anticancer activity [104,105].

Whereas *cis*-DDP is believed to act within the cell, some platinum-containing compounds appear to act on the cell membrane such as the so-called "platinum blues" [70,71]. Thus, the precise mode and site of activity may vary.

Despite the unquestionable success story of cisplatin, limitations remain including the powerful toxic side effects [103]. These toxic side effects include gastrointestinal problems such as acute nausea, vomiting and diarrhea; occasional liver dysfunction; myelosuppression involving anemia, leukopenia and thrombocytopenia; nephrotoxicity, and less frequently cases of immunosuppression, hypomagnesia, hypocalcemia, and cardiotoxicity [69]. The most serious side effect is damage to the kidney [13,106,107]. Much of the administered *cis*-DDP is filtered out of the body within a few hours, exposing the kidneys to bursts of high concentrations of platinum. The rapid rate at which the kidneys filter the platinum from the blood is believed to be responsible for the kidney problems. Another problem is the cumulative and irreversible hearing loss.

5 Structure-Activity Relationships

The topic of structure-activity relationships is complex and not fully defined for anticancer activity and is unknown for its ability to inhibit viruses. Most strategies employing polymeric derivatives of cisplatin are based in part on the assumption that the platinum-containing polymer will act similarly to cisplatin itself. However, it is likely that at least some of these polymeric drugs act in other ways to inhibit cancer growth. Although this complicates describing overall strategies, this may be an advantage because they would operate in another manner to complement cisplatin as a drug. Thus they may operate in several ways, allowing inhibition of cancer growth to occur via several mechanisms. Even so, it may be informative to briefly look at some structure-activity relationships established for cisplatin acting as an anticancer agent.

Although many promising products have been made, overarching structure-activity relationships are uncertain for a number of reasons, not the least of which is that different platinum-containing compounds may inhibit cancer growth in different ways. Thus, the following discussion should be considered as only one brief attempt at describing general structure-activity relationships. First, there should be two available anionic leaving groups such as chloride, bromide, or oxalate. Bidentate chelating groups such as dicarboxylate dianions, except for the especially labile malonato ligand, are often preferable to monodentate ligands because of their superior ability to remain intact in the bloodstream. Complexes with more labile groups such as the nitrate ion hydrolyze too rapidly for in vivo use, whereas ligands such as the cyanide ion bind the platinum too tightly impairing its activity [9,13,85,103]. Further, such complexes should have the *cis* geometry and be neutral with relatively inert amine or nitrogen donor groups. The neutrality of the molecule is believed to allow the platinum-containing drug to more easily traverse the cell membrane. The amines should be primary or secondary amines allowing for hydrogen-bonding to occur.

The relatively high Pt–N bond strength results in tight bonding of the amine, or related, ligands whereas the leaving groups such as chlorides and carboxylate anions are more weakly bonded and are readily replaced by other nucleophiles. A number of aqua complexes are formed with these aqua structures susceptible to replacement. When the pH is greater than 6.0, chloride displacement by the hydroxyl anion is favored leading to complexes containing the hydroxo group which is a relatively poor leaving group.

6 Arguments for Cisplatin-Derivative Drugs

Since the discovery by Rosenberg [88] that cisplatin is an effective anticancer drug, large synthesis and evaluation programs have aimed at the creation of cisplatin derivatives that show greater and more wide-spread activity against cancer but with

lowered toxicity. More recent activity has focused on the construction of platinum-containing homing compounds that act specifically at the desired cancer site. For many decades now, oncologists worldwide have taken advantage of the cytotoxic action of a great variety of drug systems in the fight against cancer. However, despite undisputed successes in cancer chemotherapy, particularly in combination with surgery and other treatment modalities, numerous important pharmacological deficiencies of anticancer drugs have been well recognized in the medical fraternity. Most drugs lack cell specificity, failing to discriminate between normal and cancer cells. Thus, they tend to cause severe and dose-limiting systemic toxicity. As extraneous agents, they immediately expose themselves as targets for scavenger proteins or as substrates for glomerular filtration and first-pass liver metabolism. As a consequence, serum residence times are commonly short, predominant fractions of administered doses are prematurely excreted (and wasted in the process), and bioavailability (concentration in the target tissue) is generally low. Many drugs are polar, charged, or salt-like. Therefore, they are poor substrates for membrane penetration, intracellular trafficking, and cell entry by the passive diffusion mechanism common to neutral and nonpolar compounds. In addition, drugs possessing poor solubility in aqueous media are sluggishly and incompletely dissipated in the central circulation and become easy targets for the reticuloendothelial system. Lastly, and most importantly, acquired drug resistance, which gradually builds up in the target cells after initially successful chemotherapy, is a relatively common phenomenon requiring premature treatment termination. The overall result of these shortcomings is a narrow therapeutic window and grossly limited overall chemotherapeutic effectiveness.

One approach to circumvent these deficiencies is to convert the active agent into some form of prodrug that will encounter minimal interference by scavenger mechanisms. This prodrug will be able to cross intercellular membranes and approach the target site, which in cancer chemotherapy means being able to breach the lysosomal compartment of the cancerous cell. One such group of prodrug involves the presence of the platinum-containing agent in polymers.

7   Arguments for Polymeric Drugs

Polymers can act as carriers or drugs or some combination of these two extremes. This topic is more fully discussed elsewhere. As carriers, polymers can be designed that contain many positive elements including so-called homing devices as well as the "bullet." The polymer can also be modified to achieve water solubility, desired control release kinetics, a balance of hydrophilic and hydrophobic character, and be nontoxic and nonimmunogenic. Its bulk helps to shield the drug temporarily from attack by serum proteins giving extended serum circulation half-lives. An often cited molecular weight range is from 25,000 to 80,000 as this will retard premature renal excretion while minimizing toxic effects as occasionally shown by high molecular weight polymers.

Using a pinocytotic cell entry mechanism, the carrier-attached drug, even if polar or charged, will be transported into the intracellular space, thereby overcoming possible influx inhibition, or efflux acceleration, mediated by certain well-defined drug resistance mechanisms. The process is expedited by the presence of potentially cationic moieties, such as *tert*-amino groups. Cationic sites in a polymer are known to facilitate pinocytosis while at the same time increasing affinity for the neoplastic cell, which in many cancers is negatively charged. Lastly, the enhanced permeation and retention (EPR) effect associated with macromolecules, in contrast to small compounds, provides preferred distribution of polymers to tumorous tissue. As a consequence, conjugate accumulation in the tumor is favored over that in healthy tissue. The overall result is reduced systemic toxicity and enhanced bioavailability. The drug-containing polymer can also act as a drug itself.

It is hoped that inclusion of the cisplatin-like moiety into a polymer will achieve the following:

1. It will limit movement of the biologically active drug. Because of their size, polymers are not as easily passed through membranes present in the body. Cisplatin itself is rapidly excreted from the body causing the kidney and other organs to be exposed to high concentrations of platinum. Polymers with chain lengths of about 100 units and greater typically are unable to move easily through biological membranes. Restricted movement may prevent a build-up in the kidneys and other organs thereby decreasing renal and other organ damage. Further, the platinum from polymers could be released slowly, reducing the exposure of organs to large concentrations of platinum-containing complexes.
2. It may enhance activity through an increased opportunity for multiple bonding interactions at a given site (e.g., chemical bonding, hydrogen bonding, hydrophobic interactions).
3. It should increase delivery of the bioactive moiety and decrease toxicity. In aqueous solutions, cisplatin hydrolyzes with a reaction half-life of nine hours at room temperature or 2.4 hours at 37 °C. Cisplatin hydrolyzes in the body forming a wide variety of platinum-containing agents, none of which is as active as cisplatin itself and most of which exhibit increased toxicity to the body. Formation of these hydrolysis products increases the amount of platinum complex that must be added to effect desired tumor reduction. Consequently, this increases the amount of platinum complexes that must be processed by the body. The polymeric structure should also shield the platinum moiety from unwanted hydrolysis increasing the concentration of platinum in the beneficial form that is retained in the body thus permitting lower effective doses of the drug to be used. The nature of the more hydrophobic polymer chain should also act to protect the platinum moiety from ready attack by water.
4. It should by-pass the cell's defense system. The cell's defensive response is armed as a result of invasion of other chemo drugs. Recent studies are indicating that introduction of chemo drugs into cells causes the buildup of "housekeeping" proteins that are rather general in their ability to select and remove foreign compounds present in the cell. This may be a principal reason why chemo treatments

lead to resistance to drugs, even to drugs that have not been previously used. It is possible that the polymeric nature of the platinum carriers will discourage the housekeeping proteins from removing them allowing the polymers to function as anticancer drugs under conditions where smaller platinum-containing drugs are not successful.

8 Polymer Synthesis

The polymers were synthesized by simply adding equal molar aqueous solutions of potassium tetrachloroplatinate II and the diamine-containing reactant with the resulting polymer captured as a precipitate. Polymer is formed in several minutes to several days depending on the reactivity of the Lewis base. The polymers can be made in gram to larger quantities as needed employing simple equipment and commercially available reactants. Thus, the polymers are ideally suited for commercialization.

9 Antiviral Activity

Some cancers are believed to have a viral relationship. As such it is informative to look at the viral response to some of the platinum polymers.

Tetramisole is an antihelmentic which acts on the cyclic nucleotide phosphodiesterases. It actually consists as a combination of optical isomers, the most active one being levamisole. Levamisole was the first synthetic chemical that exhibited immunomodulatory properties. It appears to restore normal macrophase and T-lymphocyte functions.

Cisplatin polymer analogs, made through reaction of tetrachloroplatinate with tetramisole, were tested for their ability to inhibit EMC-D viruses that are responsible for the onset of juvenile diabetes symptoms in ICR Swiss male mice [109]. Briefly, the mice were treated with 1, 5, and 10 mg/kg. Doses of 1 and 5 mg/kg decreased the severity and incidence of virus-induced diabetes in comparison to untreated mice. In another series of tests, doses of 1 and 10 mg/kg were administered 1 day prior to injection of the virus but here there was an increase in the severity and incidence of virus-induced diabetes. Other studies were undertaken showing that the polymer showed different activity profiles than the tetramisole (Scheme 8.29), itself.

Methotrexate is a folic acid antagonists that indirectly suppresses the synthesis of purine and is particularly effective in rapidly proliferating cell populations such as cancer. It depresses the primary and secondary antibody response, the homograft reaction, the graft-versus-host response, and the development of hypersensitivity. Methotrexate is used in the treatment of certain cancers. It is very toxic in long-term use, especially to the liver. The toxic effect of methotrexate is reversed by use of

folinic acid. This "finding" led to the development of the "rescue technique" where the most rapidly dividing cells are killed and the other cells are left unchanged.

The polymers were synthesized by simply adding equal molar aqueous solutions of potassium tetrachloroplatinate II and the methotrexate with the resulting polymer (Scheme 8.30) captured as a precipitate.

In a study related to that carried out employing tetramisole, a methotrexate polymer (Scheme 8.30) was similarly tested. In the initial study female mice were treated. Generally, only male IRC Swiss mice develop diabetes-like symptoms. Female mice must first be treated with testosterone before they can develop diabetes-like symptoms. The mice were divided into 3 groups all of which received injections of the polymer (0.5 cc IP of polymer solution containing 6.4 mg/kg). Groups I and II were treated with testosterone 1 week later. On day 8, group 1 was again given a second 0.5 cc intraperitoneal (i.p.) inoculation of the polymer solution. On day 9, all groups received 1×10^4 pfu (plaque forming units) of EMC-D virus. On day 17, all mice were given a 1 hour glucose tolerance test. The glucose
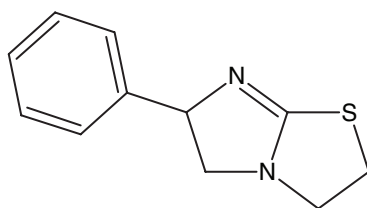
Tetramisole
29

Scheme 8.29 Tetramisole
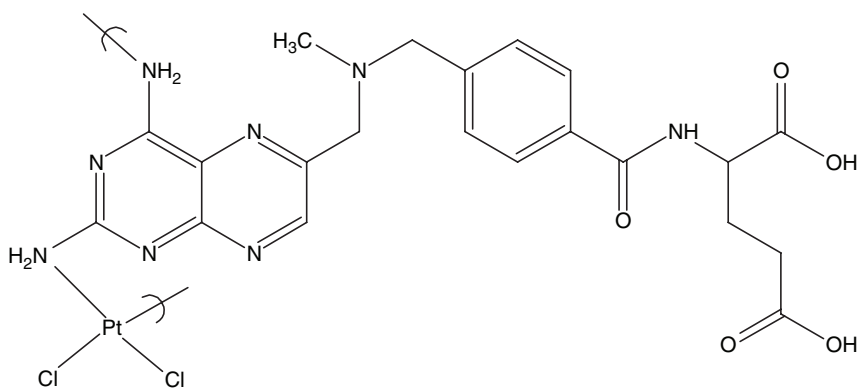
30 Methotrexate Polymer

Scheme 8.30 Methotrexate Polymer

level for groups I and III were similar and significantly below the level for the diabetic mice in group II. This is consistent with the polymer effectively blocking the diabetogenic effects of the virus. Further, other results from this study were consistent with this strain of female mice being susceptible to developing diabetes-like symptoms even without the testosterone treatment.

A related study was carried out except using male mice. Here, again, the polymer (Scheme 8.30) showed a greater positive effect on the control of diabetes than either of the reactants themselves. The glucose levels were near those of non-infected mice for the polymer-treated mice. Again, the incorporation of both the platinum and methotrexate into a polymer was the effective agent and not either of the drugs themselves. These two experiments are related to generation of a vaccine that can be employed to prevent onset of β-cell damage by RNA viruses.

The third experiment focused on treatment subsequent to viral infection [108,109]. The polymer was 100% effective in viral control with delivery of the polymer (Scheme 8.30) 1 day after the mice were infected. In summary, the methotrexate polymer (Scheme 8.30) is an effective antiviral agent against at least the EMC RNA virus.

Recently, we looked at the ability of methotrexate, tetrachloroplatinate, a physical mixture of methotrexate and tetrachloroplatinate and a methotrexate-platinum polymers to inhibit various viruses. A related study employing tilorone and a tilorone derivative and cisplatin polymeric derivatives was carried out. The results will be briefly reported later. For these studies each cell line is especially chosen to be compatible to support growth for the particular virus. DSC-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 virus 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. The other viruses are all DNA virus and the activity against different DNA viruses must be studied individually. Vaccinia is responsible for small pox; herpes simplex is responsible for at least 45 million infections yearly in the United States, or 1 out of 5 adolescents and adults; and varicella zoster is responsible for chickenpox and shingles.

The cell lines chosen for viral replication studies are cancer cell lines so that a measure of the ability of the test agents to inhibit cell growth is obtained. Each cell line is especially chosen to be compatible to support growth for the particular virus. The cell lines are all transformed cell lines.

Table 8.1 contains the GI_{50} values for these compounds for the various cell lines in μg/ml. Viral replication studies are carried out at concentrations where cell death is less than 5%.

For comparison, the GI_{50} for cisplatin for L929 cells is 50 μg/ml. Thus, all of the tested compounds except for the tetrachloroplatinate exhibit GI_{50} values less than that of cisplatin, and with the exception to the tetrachloroplatinate exhibit similar abilities to inhibit cell growth.

Table 8.1 Toxicity of methotrexate-related compounds to cancer cell lines

Compound	GI$_{50}$ (microgram/mL)			
	L929 Cells	143 Cells	Vero Cells	BS-C-1 Cells
K$_2$PtCl$_4$	375	275	225	225
Methotrexate	15	10	12	10
Polymer	10	10	10	12
Methotrexate Mix	12	8	10	10

Table 8.2 Inhibition concentrations (µg/ml) for the tested compounds

Tested Compound →	K$_2$PtCl$_4$	Methotrexate	Polymer mixture	
Reovirus ST3				
GI50	–	–	–	–
GI100	–	–	–	–
GI50*	–	–	–	–
GI100*	–	–	–	–
Vacinia WR				
GI50	–	–	–	–
GI100	–	–	8	–
GI50*	–	–	4	–
GI100*	–	–	–	–
HSV-1				
GI50	–	2	3	2
GI100	–	8	6	4
GI50*	–	2	3	2
GI100*	–	8	3	2
VZV				
GI50	–	3	4	4
GI100	–	8	4	6
GI50*	–	3	2	2
GI100*	–	8	2	3

*Based on the amount of methotrexate

Tables 8.1 and 8.2 give the results of preliminary studies involving the ability of the various polymeric drugs to inhibit the DNA and RNA associated viruses. The results are presented as means of four experiments using duplicate samples in each experiment.

Using a plaque reduction assay, the ability of each compound to prevent viral growth is summarized in Table 8.2.

The tetrachlorate is essentially inactive against the tested viruses. Methotrexate, the physical combination of tetrachloroplatinate, and the polymer all exhibit good activity against HSV-1 and VZV, both DNA viruses whose genome replication occurs in the nucleus with some activity against vaccinia virus—a DNA virus with

cytoplasmic DNA replication—and no activity against reovirus—a dsRNA virus with cytoplasmic replication. The polymer and physical mixture show good activity at lower concentrations than the methotrexate itself. Table 8.3 also contains the GI_{50} and GI_{100} for the various compounds including columns based on the amount of methotrexate itself because much of the activity appears to come from the presence of the methotrexate and little if any from the tetrachloroplatinate. It is seen that the polymer generally exhibits the lowest concentrations for inhibition consistent with the greater ability of the polymer to inhibit the tested viruses. Polymer activity may result from controlled release of the methotrexate, through the polymer itself acting as a drug, or some combination of these two extremes.

In summary, methotrexate, the mixture, and the polymer are all active against HSV-1 and VZV viruses whereas the tetrachloroplatinate showed little or no activity against any of the tested viruses. The best inhibition, that is inhibition at the lowest concentration, was found for the polymer and the mixture consistent with there being some cooperative effect of the methotrexate and platinum moieties. It is possible that the mixture of tetrachloroplatinate and methotrexate may have formed polymer when mixed together in solution consistent with the similar findings found for the mixture and polymer.

A similar study was carried out employing tilorone (Scheme 8.31) and a tilorone derivative. It is known that a molecular complex of tilorone and RNA exhibit an antiviral effect similar to that of polynucleotide interferon (IFN) inducers such as poly(I)-poly(C), larifan, and ridostin [110]. It is possible that the current responses are related to this.

Tilorone, 2,7-bis[2-9diethylamino)ethoxy]-9H-fluorene-9-one, is the first recognized synthetic small molecule structure 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.

Structures of the platinum polymers are presented in Schemes 8.32 and 8.33. Each is a *cis*-derivative obeying the *trans*-effect.

The toxicity of the tilorone polymers to the various cell lines is given in Table 8.3 in ng/ml. Both compounds show similar toxicities to the cancer cell lines tested but not to the Balb 3T3 cells which are partially transformed cells. Again, for comparison, cisplatin shows a GI_{50} of 50,000 ng/ml, over 250 times that of either platinum-tilorone polymer.

Table 8.4 gives the results of preliminary studies involving the ability of the Pt-tilorone polymeric drugs to inhibit DNA and RNA associated viruses.

Table 8.3 Toxicity of tillorone compounds to various cell lines

Compound	GI_{50} (nanogram/mL)				
	L929 Cells	143 Cells	Vero Cells	BS-C-1 Cells	Balb 3T3 Cells
Pt-Tilorone 11,567	225	200	175	200	10,000
Pt-Tillorone	180	125	175	200	500
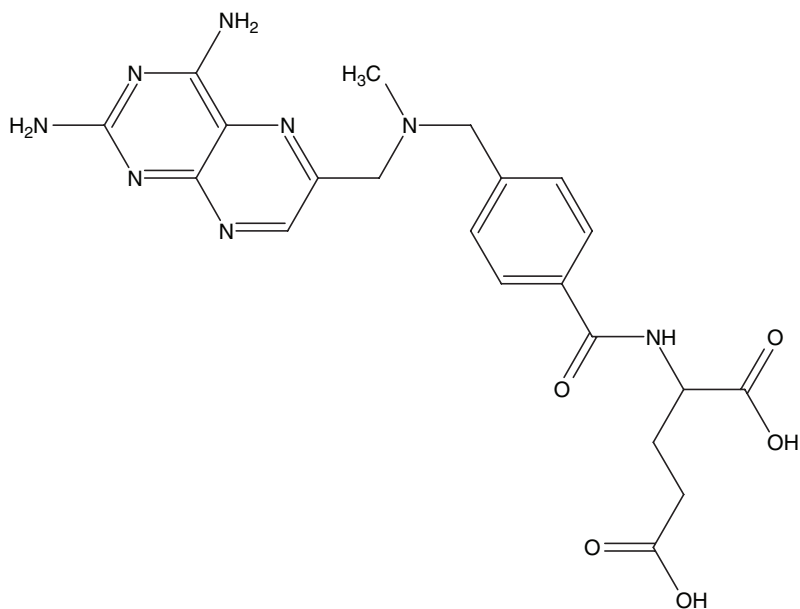
31 Tilorone

Scheme 8.31 Tilorone
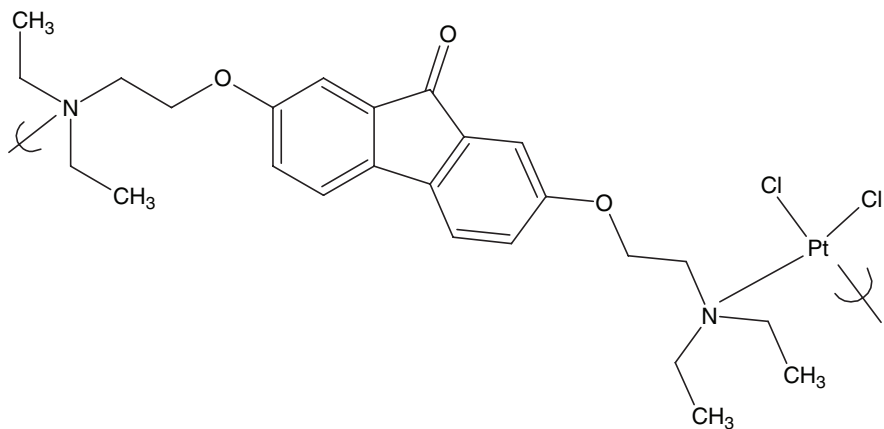
32 Product of potassium tetrachloroplatinate II and tilorone.

Scheme 8.32 Product of potassium tetrachloroplatinate II and tilorone

Both of the tillorone polymers exhibited good inhibition of all of the tested viruses at low concentrations. For comparison, the organotin polymers of norfloxacin and ampicillin showed good inhibition of these same viruses at a little higher concentrations,
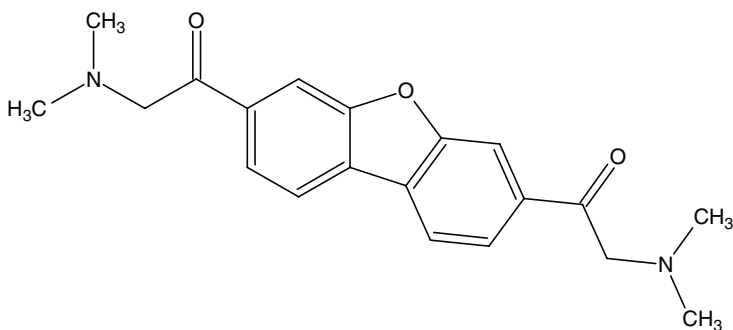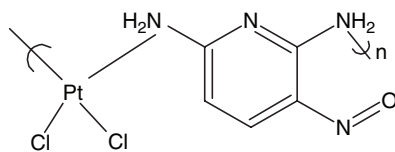
33 Product of potassium tetrachloroplatinate II and tilorone 11,567.

Scheme 8.33 Product of potassium tetrachloroplatinate II and tilorone 11,567

Table 8.4 Plaque-reduction assay results for the platinum-tilorone polymers

Compound	GI_{99+} Concentration (ng/ml)			
	Reovirus ST3	Vaccinia WR	HSV-1	BS-C-1
Pt-Tilorone 11,567	200	150	150	150
Pt-Tilorone	125	100	125	100

34

Scheme 8.34

within the range of less than 1 to 2 mg/ml, whereas the tilorone compounds showed good activity at 0.2 mg/ml as noted above.

In summary, the tilorone polymers inhibit both RNA and DNA viruses and deserve further consideration as antivirial agent in the war against viruses and possible bioterrorism involving viruses. Further, they show good inhibition of virus replication in both transformed cell lines but also in normal cell lines, a condition that better mimics antiviral therapy of humans.

A number of platinum polyamines were tested for antiviral activity in tumor cells [111]. For instance, the polymer from tetrachloroplatinate and 2,6-diamino-3-nitroso-pyridine (Scheme 8.34) which exhibited a cell differential ratio of 3.4, was tested at a concentration of 2.2 µg/ml on L929 cells infected with

Encephalomyocarditis (EMC) virus, strain MM. A virus reduction of about 25% was seen. This is considered to be a moderate antivirial response.

In general, agents capable of inhibiting one RNA virus will inhibit other RNA viruses but each DNA virus must be evaluated separately. The platinum polyamines were studied against RNA viruses. The behavior toward RNA viruses was varied with some showing little activity but the majority showing inhibition of viral replication at polymer concentrations below which tumoral inhibition is found ($<1\,\mu g/ml$).

The effect of platinum polyamines on the transformation of 3T3 cells by SV40 virus was also studied [111]. In summary, these polymers showed no effect on the transformation process.

10   Conclusions

Polymeric derivatives of cisplatin inhibit the replication of a number of important viruses. The results are consistent with this family of compounds showing promise as a new family of antiviral agents. Further, the results indicate that additional study of members of this family as antiviral agents is warranted.

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