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Review

DNA encapsidation as a target for anti-herpesvirus drug therapy

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Abstract

The current repertoire of approved anti-herpesviral drugs consists primarily of nucleoside analogues that inhibit viral replication by targeting the virus-encoded DNA polymerase. This class of agents has been critical in controlling infections by herpes simplex, varicella zoster, and cytomegalovirus. However, because nucleoside analogues share a similar mechanism of action, treatment options are limited once resistance develops. This becomes an important medical issue with respect to the treatment of disease caused by resistant viral strains, particularly in immunocompromised individuals. Furthermore, several of the currently available therapies can result in mild to severe side effects making the discovery of less toxic drugs desirable. Efforts over the last decade have focused on the identification and development of improved therapies including less toxic compounds with novel mechanisms of action. Here we review the progress that has been made in targeting the DNA packaging and encapsidation process as a novel target for chemotherapy. Several recently identified compounds may warrant further development as a medically important group of herpesviral encapsidation inhibitors.

Keywords: Herpesviruses; Antiviral; Encapsidation; Portal

1. Introduction

The human pathogenic herpesviruses are a family of double stranded DNA viruses that infect a variety of hosts (Whitley and Gnann, 1993; Weir, 1998). Genetic relatedness, host range, the virus life cycle and the cell type in which a virus establishes and reactivates from latency, form the basis for classification as either an alpha, beta or gamma herpesvirus (Alba et al., 2001; Roizman, 1996).

The alphaherpesvirus herpes simplex virus type 1 (HSV-1) and its close relative, herpes simplex virus type 2 (HSV-2), initially infect mucosal epithelial cells and establish latency in cells of neuronal origin (Whitley and Roizman, 2001). HSV-1 is the predominant cause of cold sores whereas HSV-2 primarily causes genital herpes (Whitley and Gnann, 1993; Stanberry et al., 1999; Simmons, 2002). Varicellla zoster virus (VZV), the third human alphaherpesvirus, causes a common and generally self limiting primary infection, chickenpox, while reactivation of latent VZV from neurons results in herpes zoster or shingles, a painful and debilitating disease (Cohen et al., 1999; Tenser, 2001).

The betaherpesviruses, including human cytomegalovirus (HCMV) and human herpes viruses type 6 and 7 (HHV-6, HHV-7), characteristically infect and establish latency in lymphocytes and cells of the monocyte/macrophage lineage (Rice et al., 1984; Frenkel and Wyatt, 1992; Zhuravskaya et al., 1997; Black and Pellett, 1999). HCMV is usually asymptomatic as a primary infection in healthy people, but can significantly impair the neuronal development of a fetus when the mother is infected during pregnancy. HCMV has been shown to cause a high level of morbidity and mortality in immunocompromised populations such as transplant, cancer and AIDS patients (Patel and Paya, 1997; Fishman, 1999; Griffiths et al., 2000; Sissons and Carmichael, 2002). HHV-6 and HHV-7 typically cause infections that occur within the first 3 years of life and while HHV-7 has yet to be directly linked to any specific disease, HHV-6 appears to be the causative agent of roseola (De Araujo et al., 2002). A significant role for reactivated HHV-6 and HHV-7 in causing opportunistic disease following organ transplantation

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is likely (Kimberlin, 1998; Gentile, 2000; Singh, 2000; Dockrell and Paya, 2001).

Lastly, Epstein-Barr virus (EBV) and human herpes virus type 8 (HHV-8) are the only described human gammaherpesviruses. EBV infects B-cells and causes infectious mononucleosis and Burkitt's lymphoma (Okano and Gross, 2001; Cesarman, 2002). HHV-8 is strongly associated with the development of Kaposi sarcoma, a common opportunistic infection of AIDS patients (Ensoli et al., 2001; Schulz et al., 2002).

Although some type of therapy exists for many of the above mentioned human herpesviruses, limitations due to specificity, bioavailability, toxicity and/or the development of resistance merit the continued discovery and development of new and improved drug candidates.

2. Current therapies

The ability of herpesviruses to establish and reactivate from a latent state in their host means that therapies targeted to merely inhibit viral replication are not sufficient to eradicate infection. Until a better understanding of latency is obtained, replication inhibitors will continue to play an indispensable role in limiting recurrent outbreaks, and when used prophylactically, in reducing the frequency of outbreaks (Gold and Corey, 1987; Kuzushima et al., 1992; Spruance, 1993; Wall et al., 1999; Ormrod et al., 2000; Schwartz and Holland, 2000). Current therapy for herpesviruses consists primarily of DNA polymerase inhibitors (Elion, 1983; Frank et al., 1985; Matthews and Boehme, 1988; Chrisp and Clissold, 1991) including acyclovir (ACV), ganciclovir (GCV), penciclovir (PCV), brivudin (BVDU), cidofovir (HPMPC), and foscarnet (PFA). These drugs target the viral polymerase, the enzyme responsible for viral genomic replication, and fall into two chemical classes: nucleoside analogues (De Clercq, 1984; Littler et al., 1992) and pyrophosphate analogues (Eriksson et al., 1982; Littler et al., 1992).

Nucleoside analogues are substrates for the viral polymerase. Antiviral activity of nucleoside analogues is dependent on the metabolic conversion to their respective tri-phosphate form via three separate phosphorylation events, the first of which is catalyzed by viral phosphotransferases with the second and third catalyzed by cellular enzymes. Incorporation of nucleoside analogues into the nascent DNA chain blocks DNA elongation. Certain of these drugs have substantial side effect profiles and/or are poorly bioavailable (Table 1) (Haynes et al., 1996; Tomicic et al., 2002). Oral administration of the prodrug-form of ACV, GCV or PCV (valacyclovir, valganciclovir, and famciclovir, respectively), results in higher plasma levels and increased bioavailability of active drug. Development of the orally bioavailable forms has proven to be extremely useful and convenient when treating HSV and CMV disease. It is not surprising that the prodrug forms retain target specificity identical to their parent drug and hence are not useful in cases where viral disease is a result of resistance to ACV, GCV or PCV. Furthermore, and particularly in the case of valganciclovir, administration of the prodrug form does not eliminate the occurrence of unwanted side effects associated with the parent analogue (Smiley et al., 1996; Reusser, 2001).

Although not approved for use in the US, BVDU, a pyridine-containing nucleoside analogue, has proven to be a potent inhibitor of alphaherpesviruses (De Clercq and Walker, 1984) and is particularly effective for VZV. BVDU shows an excellent toxicity profile and is orally bioavailable. Currently, BVDU is approved for treating VZV infections in Germany. Although proven to be extremely successful in the treatment of herpes zoster (Wutzler et al., 1995), further worldwide approval has likely been slowed by the fact that sorivudine, a close analogue of BVDU, was linked to several deaths in Japan after co-administration with the anticancer drug 5-fluorouracil (Diasio, 1998).

The two currently available therapeutic alternatives include cidofovir, a nucleoside phosphonate requiring only two cellular phosphorylation events to become an active drug (Neyts and De Clercq, 1994; Cihlar and Chen, 1996), and foscarnet (phosphonoformic acid), an orthophosphate mimic that directly inhibits the viral polymerase (Tyms et al., 1987; Crumpacker, 1992). Both drugs are poorly bioavailable and foscarnet is extremely toxic (Table 1) (Sjovall et al., 1988; Deray et al., 1989). Hence, these compounds are often used as a second choice when resistance to one of the nucleoside analogues is observed.

Lastly, fomivirsen, an antisense oligonucleotide-based therapy designed to inhibit HCMV immediate-early gene transcription, requires delivery via intra-ocular injection for the treatment of HCMV-induced retinitis (Leeds et al., 1997; Perry and Balfour, 1999).

3. Clinical need for new drugs against novel targets

Generally speaking, the use of therapeutic drugs is associated with the development of resistance (Table 1) (Gilbert et al., 2002) and in immunosuppressed individuals, this can often lead to treatment failure. For the polymerase inhibitors cidofovir and foscarnet, resistance appears in the polymerase gene itself and for the nucleoside analogues additional resistance mutations appear in the viral phosphotransferase genes which are responsible for the mono-phosphorylation of nucleosides, i.e. thymidine kinase (TK) for HSV and VZV (Darby et al., 1981; Elion, 1983) and UL97 for HCMV (Littler et al., 1992).

In immunocompromised patients, the development of resistant viruses can result in severe disease capable of spreading systemically and becoming fatal (Gateley et al., 1990; Darville et al., 1998; Read et al., 1998; Oram et al., 2000). Hence, it is clearly recognized that alternative therapies are needed (Snoeck and De Clercq, 2002; Wathen, 2002). Resistance to a drug can be circumvented by using (i) new

Table 1
Properties of currently marketed anti-herpesviral drugs

Antiviral	Route[a]	Virus[b]	Resistance[c]	Potential side effects
Acyclovir	IV, oral, topical	HSV-1 and -2, VZV		Headache, nausea
Valaciclovir	Oral	HSV-2, VZV	+	Hemolytic uremic syndrome[d]
Penciclovir	Topical	HSV-1		Neurotoxicity including delirium/coma[d]
Famciclovir	Oral	HSV-2, VZV		
Ganciclovir	IV	HCMV	+	Myelosuppression resulting in neutropenia
Valganciclovir	Oral	HCMV		Thrombocytopenia
Brivudin	Oral, topical	VZV	+	Potential for serious drug intereactions with fluorouracil
Cidofovir	IV	HCMV	+	Nephrotoxicity, proteinuria Ocular toxicity
Foscarnet	IV	HCMV		Nephrotoxicity, intraglomerular deposition, necrosis
			+	Electrolyte disturbances[e] Epidermal necrolysis[f] Hematoxicity in combination with AZT
Fomivirsen	IO	HCMV	+[g]	Intraocular administration only Inflammation

[a] Common route of administration.
[b] Viral target.
[c] Resistant virus isolates observed in clinical samples.
[d] Rarely reported.
[e] Can be controlled for during administration of drug.
[f] When used topically.
[g] Observed in vitro in laboratory setting.

compounds that prevent viral replication by inhibiting an entirely different viral target; or (ii) by employing alternate ways to attack an already targeted enzyme function by virtue of a novel compound binding site. In this latter class, non-nucleoside polymerase inhibitors were recently described which prevent replication of a broad spectrum of herpesviruses (Brideau et al., 2002; Knechtel et al., 2002; Wathen, 2002).

4. Herpesvirus targets

Herpesviruses have some of the largest genomes among viruses and hence provide a wealth of therapeutic targets. Recognizing the clinical need for new therapies, researchers have made significant progress in identifying novel virus specific targets.

One such target, the helicase/primase complex, has a role early in the viral life cycle and is known to be an essential component of the replication machinery (Crumpacker and Schaffer, 2002). Two different chemical classes of helicase/primase inhibitors with promising in vitro and in vivo results are currently in development: BAY 57-1293 (Betz et al., 2002; Kleymann et al., 2002) and BILS 179 BS (Crute et al., 2002).

The UL97 phosphotransferase of HCMV, which is responsible for the mono-phosphorylation of ganciclovir, has become a drug target in its own right (Littler et al., 1992). Although the exact role of the kinase activity of UL97 has not been clearly defined, inhibitors that reduce kinase activity point to a critical role early in the HCMV replication cycle for the UL97 protein (Wolf et al., 2001). Benzamidizole compounds that inhibit the UL97 kinase activity are active against viruses containing ganciclovir-resistant UL97 mutations (McSharry et al., 2001; Biron et al., 2002). Benzamidivir has completed phase I clinical trials and has been shown to have a good safety profile (Naesens and De Clercq, 2001; Lalezari et al., 2002).

Several targets that have a role later in the infection cycle have shown to be promising candidates for antiviral therapy. Inhibitors of the viral proteases of HSV and HCMV have been the focus of many labs (Flynn et al., 1997a,b; Holwerda, 1997; Patick and Potts, 1998; Waxman and Darke, 2000). The herpesviral protease cleaves the capsid scaffold protein, an essential step in the maturation of viral capsids (Shieh et al., 1996; Gibson and Hall, 1997). Although many molecules have been proposed as drug candidates (Flynn et al., 1997a,b; Matsumoto et al., 2001), none have had ideal drug properties and hence, their development has all but been abandoned.

Lastly, proteins (Fig. 1) that play an essential role in the DNA encapsidation process have become promising novel targets and are the focus of the remainder of this review.

5. Herpesvirus DNA encapsidation

Virions of the *Herpesviridae* contain four main components: the DNA genome, the capsid, the tegument and the viral envelope. Capsids are assembled and filled with DNA in the nucleus. The tegument and envelope are added to the nucleocapsids upon egress from the nucleus and cell (for a

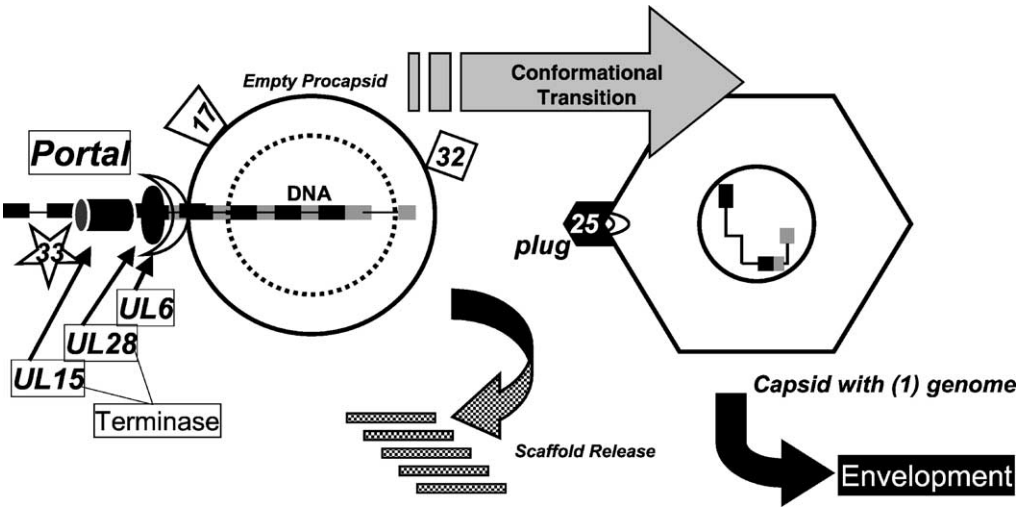

Fig. 1. Pictorial view of DNA encapsidation. The specific functions of the HSV-1 gene products involved in the DNA encapsidation process are currently being defined and information and reagents are emerging to allow for further dissection of the herpesviral DNA-cleavage and packaging machinery. The localization of capsid proteins and/or capsids appears to be mediated by the UL32 and UL17 gene products early in the process. These proteins appear to be required for the proper translocation of capsid proteins to replicative sites within the nucleus. The UL15 and UL28 gene products have a potential role in the cleavage of progeny DNA, and are the presumed terminase subunits. The UL33 gene product is essential for DNA packaging, important in the localization of other proteins to replication compartments, and is a terminase-associated protein. The UL6 protein product is now thought to be the portal for viral DNA entry into the preformed procapsid. The protein encoded by the UL25 gene does not seem to play a role in the DNA cleavage event, but appears to be important in stabilizing DNA-filled capsids and may act as a vertex "plug".

review see Mettenleiter, 2002). Envelope proteins are modified during transit to the plasma membrane prior to virion release from the cell.

Our knowledge of capsid assembly and DNA packaging is continually modified through new discoveries. After incorporating the most recent advances, our current view is as follows. During viral replication, progeny DNA genomes are synthesized in the nucleus as long, branched head-to-tail concatamers (Zhang et al., 1994; Severini et al., 1996). The capsid proteins are synthesized in the cytoplasm, and are transported to the nucleus either by using their own nuclear localization signal (NLS) or by binding to another protein containing a NLS (Rixon et al., 1996; Plafker and Gibson, 1998; Koslowski et al., 1999). HSV capsid assembly is thought to start in the cytoplasm with the association of the scaffold protein precursor, pre-VP22a, with VP5, the major capsid protein. After entry into the nucleus by virtue of the pre-VP22a NLS, these dimers assemble into hexons and pentons driven by the self-association of the scaffold protein precursors (Wood et al., 1997). Concurrently, the viral maturational protease (encoded by the UL26 gene) is also thought to associate with VP5 via its C-terminus which contains the entire pre-VP22a protein sequence (Thomsen et al., 1995). The fully spherical procapsid also contains the minor capsid proteins, VP19C and VP23, which fill the spaces between the VP5 hexons and pentons (Trus et al., 1996; Newcomb et al., 2000, 2001a,b). A series of events orchestrated by the viral protease results in cleavage of the scaffold protein and the subsequent transformation of the spherical procapsid into an icosahedral shaped, B-type capsid (Fig. 1) (Trus et al., 1996; Homa and Brown, 1997). B-type capsids can be found in infected cells, and in vitro, after assembly from a mixture of five HSV-1 capsid proteins, VP5, VP19C, VP23, scaffold protein, and the protease (Newcomb et al., 1994, 1999; Tatman et al., 1994; Thomsen et al., 1994). Whether B-type capsids in infected cells are the product of failed DNA-packaging attempts or whether they are the starting point for DNA packaging is still an issue of some debate. Successful DNA packaging includes the removal of the cleaved scaffold proteins and the tight packing of one unit length of herpesviral genomic DNA, resulting in dense core C-type capsids (Fig. 1). Finally, A-type capsids have neither scaffold nor DNA.

Irrespective of whether genomic cleavage and packaging starts with pro- or B-capsids, the concatameric DNA must be "guided" into the capsid during the encapsidation process. In comparison to the more fully unraveled encapsidation process of double-stranded bacteriophages, herpesvirus encapsidation could involve analogous functions such as: (i) a capsid portal structure; (ii) proteins that recognize the ends of viral DNA; and (iii) an endonuclease plus a DNA translocase (terminase complex) (Black, 1995; Catalano et al., 1995). Seven genes have been shown to be essential in the HSV DNA encapsidation process: ULs 6, 15, 17, 25, 28, 32 and 33 (Fig. 1, Table 2) (Patel and MacLean, 1995; Chang et al., 1996; Lamberti and Weller, 1996, 1998; Patel et al., 1996; McNab et al., 1998; Salmon and Baines, 1998; Taus and Baines, 1998; Taus et al., 1998; Yu and Weller, 1998a,b; Koslowski et al., 1999; Goshima et al., 2000; Reynolds et al., 2000; van Zeijl et al., 2000; Newcomb et al., 2001a,b; Ogasawara et al., 2001; Sheaffer et al., 2001; Beard et al., 2002). These genes are conserved throughout

Table 2
Properties of the HSV, VZV, and HCMV DNA encapsidation genes and their associated gene products

HSV-1	MW (kDa)	HSV-2[a]	HCMV[b]	VZV[a]	Function	Capsid[c]	References
UL6	75	UL6 (86)	UL104 (27/36)	ORF54 (44)	Localizes to replication compartments; portal protein	B	Patel et al. (1996), Taus et al. (1998), van Zeijl et al. (2000)
UL15	79, 81, 83	UL15 (95)	UL89 (43/53)	ORF42/45 (62)	Localizes to replication compartments; putative terminase; DNA binding and cleavage activity; forms complex with UL28	B	Salmon and Baines (1998), Yu and Weller (1998a,b), Koslowski et al. (1999)
UL17	78	UL17 (82)	UL93 (25/31)[d]	ORF43 (36)	Tegument protein; localizes capsid and encapsidation proteins to replication compartments	B	Taus et al. (1998), Goshima et al. (2000)
UL25	60	UL25 (88)	UL77 (27/34)	ORF34 (46)	Role in stabilizing DNA-filled capsid; not required for DNA cleavage	A	McNab et al. (1998), Ogasawara et al. (2001)
UL28	85	UL28 (90)	UL56 (27/37)	ORF30 (49)	DNA binding; forms complex with UL15; endonuclease activity	B	Patel and MacLean (1995), Lamberti and Weller (1996), Koslowski et al. (1999), Taus and Baines (1998), Taus et al. (1998), Yu and Weller (1998a,b), Beard et al. (2002)
UL32	67	UL32 (88)	UL52 (28/35)	ORF26 (47)	Predominantly cytoplasmic; role in localization of capsids to replication compartments; zinc binding	B	Chang et al. (1996), Lamberti and Weller (1998), Taus et al. (1998)
UL33	19	UL33 (92)	UL51(32/43)	ORF25 (40)	Associates with UL15/UL28 complex; inferred role in DNA cleavage	B	Beard et al. (2002)

[a] ( ): % identity with HSV-1 protein.
[b] (/): % identity/similarity with HSV-1 protein.
[c] Predominant capsid type accumulating in corresponding gene deletion mutant.
[d] Tentative assignment based on % similarity.

the herpesvirus family (Alba et al., 2001) and for ease of reference, HSV-1 nomenclature will be used here. When any of these seven genes are deleted from the viral genome, empty capsids and un-cleaved DNA accumulate in the nucleus (Table 2). B-type capsids accumulate in all but one of the seven deletion mutants. Only UL25-deleted viruses show an accumulation of A capsids, and hence the UL25 gene product has been assigned a role in the stabilization of capsids after the DNA has entered (Catalano et al., 1995; McNab et al., 1998; Ogasawara et al., 2001).

The herpesvirus DNA encapsidation process is still understood only in general terms, and despite the unraveling of many details, the exact order of events has yet to be determined (for a review see Brown et al., 2002). Capsid protein precursors important in forming procapsids are localized to specific nuclear structures by the UL17 and UL32 proteins (Lamberti and Weller, 1998; Taus et al., 1998). Replicated DNA also localizes to nuclear replication compartments, and is probably associated with a complex of proteins consisting of the UL28, UL15 and UL33 gene products (Fig. 1) (Koslowski et al., 1999; Reynolds et al., 2000; Sheaffer et al., 2001; Beard et al., 2002). Based on evidence for their homologous counterparts in HCMV (Bogner et al., 1998; Giesen et al., 2000; Scheffczik et al., 2002), the UL15 and UL28 proteins are the likely HSV terminase subunits (Fig. 1). The HCMV terminase subunits, UL56 and UL89, have been shown to possess DNA binding activity, and to specifically recognize packaging (pac) sequences located near the ends of herpesvirus genomes. In addition, these two proteins were shown to possess nucleolytic activity and ATPase activity and hence are the most likely candidates for the terminase function implicated in the cleavage of the concatameric viral DNA to a single genome length. Recent papers by Newcomb and colleagues (Newcomb and Brown, 1994, 2002; Newcomb et al., 2001a,b) provide evidence that the HSV UL6 gene product forms the portal protein through which the DNA can enter the pro or B-capsid (Fig. 1).

6. DNA encapsidation targets

The DNA encapsidation protein complex of herpesviruses is now thought to include a heterodimeric terminase unit (Koslowski et al., 1999; Bogner, 2002; Scheffczik et al., 2002). Several classes of *Herpesviridae* inhibitors have been characterized which have a mechanism of action that appear to target the viral terminase complex (Krosky et al., 1998; Buerger et al., 2001; Reefschlaeger et al., 2001). Interestingly, although the compounds identified thus far (Fig. 2) show a similar mechanistic phenotype as a result of their inhibitory activity, the separate classes appear to act upon different viral cleavage and packaging proteins found within the terminase complex.

Two benzimidazole ribonucleosides (BDCRB, TCRB) inhibit the replication of human cytomegalovirus by inhibiting cleavage of viral DNA (Krosky et al., 1998). These inhibitors were shown to result in the accumulation of immature capsids and uncleaved DNA in infected cells. Maximal resistance to these compounds was mapped to two different encapsidation genes, UL89 and UL56, homologues of the HSV-1 UL15 and UL28 genes, respectively (Krosky et al., 1998; Underwood et al., 1998). The UL89/56 gene products are thought to make up the heterodimeric HCMV terminase complex (Scheffczik et al., 2002). The

Table 3
Cellular toxicity and efficacy of DNA encapsidation inhibitors

Compound	Major target	CC_{50} (μM)[a]	SI[b]	In vivo efficacy[c]	Clinical trial[d]	References
TCRB	HCMV	210[e]	72	(Metabolically unstable)	nd	Townsend et al. (1995)
BDCRB	HCMV	>100[e]	>140	(Metabolically unstable)	(GW 273175X[f])	Gudmundsson et al. (1997, 2000)
BAY 38-4766	HCMV	93[g]	310	HCMV hollow fibre implant model	Phase I[h]	Reefschlaeger et al. (2001)
BAY 38-4766	MCMV	63[i]	1602	MCMV-infected SCID mouse model		Weber et al. (2001)
5 Chloro-1,3-dihydroxyacridone	HSV	52[j]	13	nd	nd	Akanitapichat et al. (2000, 2002)
WAY-150138	HSV	>100[e,j]	>250	nd	nd	van Zeijl et al. (2000)
Comp 1	VZV	>36[e,j]	>50	nd	nd	Visalli et al. (2003)

nd: no data.

[a] 50% cytotoxic concentration.

[b] Selectivity index (CC_{50}/IC_{50}).

[c] Animal model testing.

[d] Testing in human subjects.

[e] Human foreskin fibroblasts (HFF).

[f] GW 273175X, a BDCRB derivative with improved pharmacokinetic properties, has yielded encouraging results in initial human studies (Boyd et al., 1999).

[g] Human embryonic lung fibroblasts (HELF).

[h] Positive results; halted in favor of new derivative.

[i] Murine embryonic fibroblasts (MEF).

[j] African green monkey kidney cells (Vero).

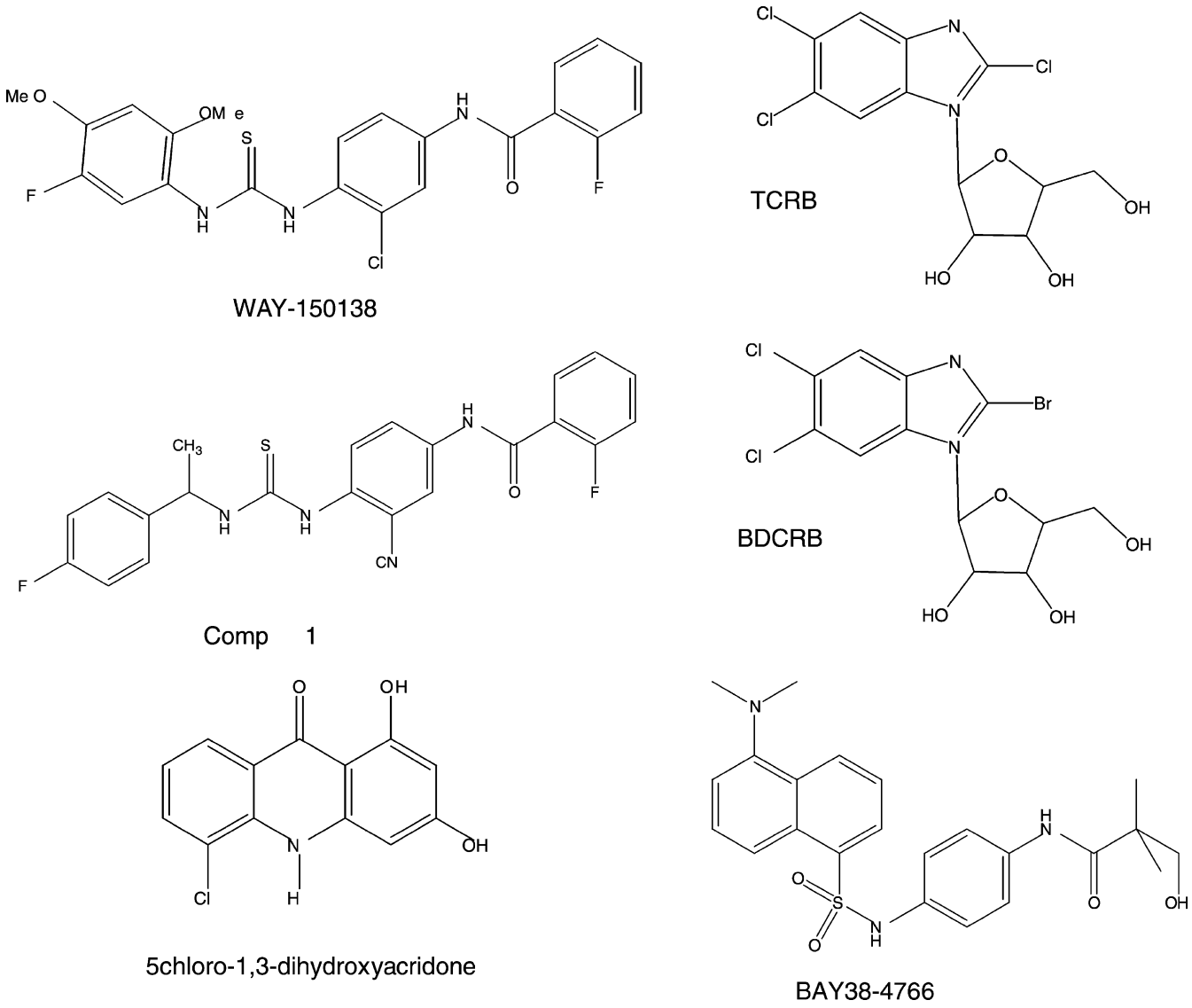

Fig. 2. Chemical structures of selected *Herpesviridae* inhibitors. WAY-150138: thiourea analogue; Comp 1: α-methylbenzyl thiourea analogue; 5 chloro-1,3-dihydroxyacridone: 1,3-dihydroxyacridone analogue; TCRB and BDCRB: benzimidazole analogues; BAY 38-4766: sulfonamide analogue.

benzimidazole compounds appear not only to be potent inhibitors but also highly specific for the terminase complex of HCMV in that previous studies showed that BDCRB readily inhibited HCMV but not murine CMV (MCMV), VZV, HSV-1 or -2 (Townsend et al., 1995).

Recently, Buerger et al. (2001) described a series of non-nucleoside inhibitors (BAY 38-4766 and associated analogues) that interfere with HCMV viral DNA cleavage and packaging via the UL89 and UL56 terminase subunits but whose molecular mode of interaction is in fact separable from the benzimidazole ribonucleosides. This BAY series of compounds differs from the previously reported HCMV terminase inhibitors in (a) the apparent lack of demonstrable viral cross-resistance to the benzimidazole ribonucleoside series (Evers et al., 2002), and (b) their broadened range of specificity (i.e. they inhibit monkey and rodent cytomegaloviruses as well as HCMV) (Buerger et al., 2001; Reefschlaeger et al., 2001; Weber et al., 2001). Furthermore, resistant viruses isolated in the presence of the BAY series harbor additional mutations outside of the UL89 and UL56 genes. Both murine and human CMV isolates resistant to the BAY compound reveal mutations in the UL104 gene (the HSV UL6 homologue; see Section 7). The data suggests that the observed UL104 mutations did not directly contribute to the resistant phenotypes of the CMV isolates. However, since the UL104 protein and the UL56 and/or UL89 proteins may form a functional complex during the DNA cleavage and packaging process, the authors speculated that UL104 mutations might compensate for potential conformational changes in altered UL56 and/or UL89 gene products. The BAY compounds have proven to be effective in vivo in a MCMV model of pathogenicity and, along with favorable pharmacokinetic and safety data, are proceeding toward clinical development (Reefschlaeger et al., 2001; Weber

Table 4
Comparative activity and selectivity of thiourea compounds

Virus	Strain	IC_{50}			
		Comp 1		WAY-150138	
		μg/ml	μM	μg/ml	μM
HSV-1	Patton	>10	>25	0.2	0.4
HSV-2	12	nd	nd	6.8	14.8
VZV	Ellen	0.26	0.63	>10	>21.7
SVV		~2	~5	nd	nd
HCMV	AD169	>7	>17	6	13

et al., 2001). Since mammalian cells presumably lack an activity for the cleavage of concatameric DNA, inhibitors of viral terminases should prove to be specific and potentially non-toxic. Selectivity indices for the currently identified DNA encapsidation inhibitors are summarized in Table 3.

7. Portal protein as a viable encapsidation target

In the double-stranded DNA bacteriophages such as lambda and T4, an encapsidation complex is assembled on the concatameric DNA and one end of the progeny genome is generated by endonucleolytic cleavage. This protein–DNA complex then binds to the preassembled procapsid (prohead) at the portal vertex and DNA is subsequently packaged and cleaved a second time to release the other end of the genome (Lin et al., 1999). The related processes that are thought to occur in eukaryotic DNA viruses are just now beginning to be elucidated, including those of the Herpesviridae. Certainly, an understanding of such mechanisms for human pathogens has the added benefit of the identification of novel targets for antiviral chemotherapy. The following section describes the recent identification of small molecule compounds that target herpesvirus portal proteins.

7.1. Activity and structure of thiourea inhibitors

Recently, the discovery of two related classes of thiourea compounds (Fig. 2) was described. One series of compounds specifically inhibited HSV-1, and to a 10-fold lesser extent HSV-2, while the other inhibited VZV (Table 4) (van Zeijl et al., 2000; Visalli et al., 2003). Interestingly, compounds from either series had little activity against other human herpesviruses, and also showed a lack of reciprocal activity within the alphaherpesvirus family (HSV versus VZV). The lack of reciprocal activity is interesting, if not surprising, considering the minimal differences between the compounds. While the VZV and HSV inhibitors have similar structures (Fig. 2), minor chemical differences appear to result in a remarkable degree of specificity for inhibition of specific alphaherpesviruses. For example, the addition of an α-methyl linker in the VZV series eliminated observable activity against HSV (Table 4). Furthermore, the amino acid similarity between HSV-1 and HSV-2 DNA cleavage and packaging proteins is ~89% (Table 2), yet there is a 10-fold difference in sensitivity to WAY-150138 (Table 2). A similar situation is observed for the VZV series in that >10-fold less activity was observed against Simian Varicella Virus (SVV) in vitro (Table 4). [Recently, the entire SVV genomic sequence was completed and showed that VZV and SVV share a high level of amino acid identity (Gray et al., 2001).] The results to date indicate a remarkable specificity for each of the series' specific viral target.

7.2. Generation of resistant viral mutants and target identification

Experiments with laboratory-generated mutants resistant to the two series of compounds described above, indicated that resistance was associated with mutations in the UL6 or ORF54 genes (van Zeijl et al., 2000; Visalli et al., 2003). The mutations that conferred VZV resistance to the thiourea compounds mapped to amino acid residues 324 and 408 of the ORF54 protein (769 amino acid polypeptide) in one resistant isolate, and to residue 407 in another resistant isolate. The mutations that conferred resistance for HSV-1 to the other class of thiourea compounds mapped to amino acid residues near the amino and carboxyl ends of the UL6 protein (676 amino acid poylpeptide). Thus, while the two different classes of structurally related thiourea compounds both appear to target homologous proteins in VZV and HSV-1, resistance appears to map to different regions of the proteins, at least for the mutants studied thus far.

For the HSV specific series, one can speculate that the two relatively distant areas of UL6 for which mutations were detected might come together in the three-dimensional structure of the protein to form interaction surfaces with the compounds. Alternatively, the N and C terminal portions of UL6 may interact in a more complex multimeric fashion with itself or with the other cleavage and packaging proteins.

The VZV ORF54 mutations conferring resistance mapped more centrally. How, if at all, these mutations relate to those observed at the distal ends of the HSV-1 UL6 protein is not known. The isolation of more resistant viruses for both classes of inhibitors may be valuable reagents to further delineate portal protein function.

7.3. Mechanism of action of thiourea inhibitors

The HSV UL6 protein (Patel and MacLean, 1995), one of the seven HSV proteins essential for viral DNA cleavage and packaging (Lamberti and Weller, 1996; Patel et al., 1996), was shown to be the likely target of the HSV specific series and the VZV series targeted the ORF54 protein, the UL6 homologue. The phenotypic effects of UL6 or ORF54 inhibition by the thiourea compounds are consistent with the genetic evidence provided by previous studies with HSV deletion mutants (Patel et al., 1996). When HSV

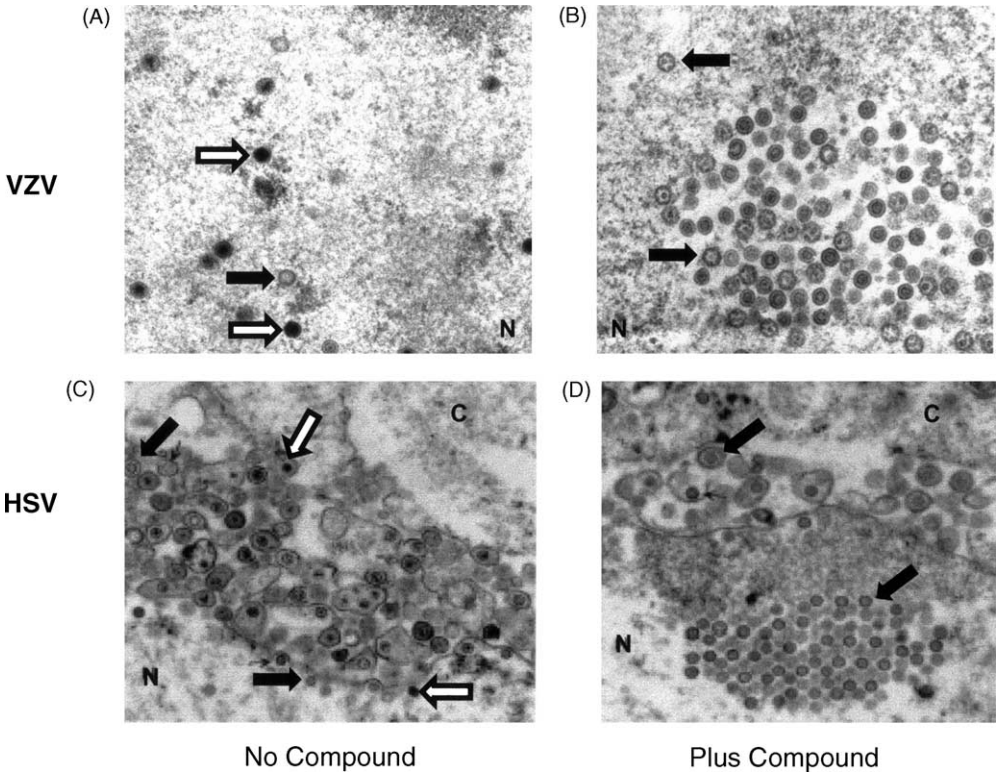

Fig. 3. Electron microscopic examination of VZV and HSV capsid morphologies of cells infected in the presence of thiourea inhibitors. Melanoma cells infected with VZV ROka (A and B) or Vero cells infected with HSV (C and D) were treated with an α-methylbenzyl thiourea (B) or a thiourea (D) analogue. Panels B and D show the absence of mature DNA filled capsids when drug is present. Filled arrows point to immature B capsids while open arrows indicate mature C-type capsids. Nucleus (N).

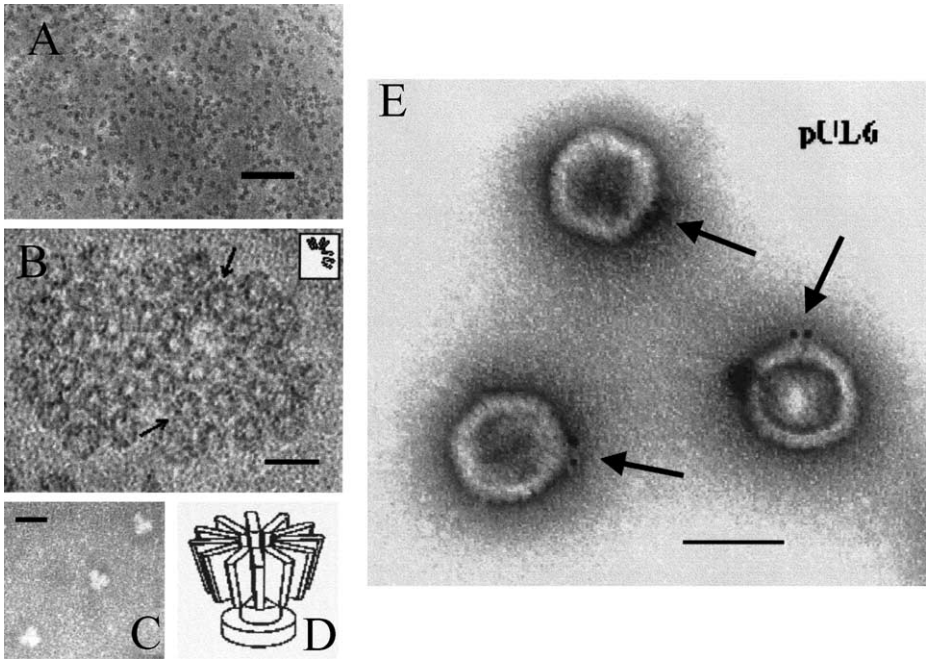

Fig. 4. Morphology of purified HSV UL6 protein and localization of the UL6 protein on the HSV capsid (Newcomb and Brown, 2002). UL6 purified from Sf9 cells appears as ring-like structures in negatively stained preparations (A and B). A Y-shaped structure reveals itself on glow-discharge grids (C). Panel D depicts a diagrammatic representation of UL6 structures derived from the EM images in panels A–C. The localization of UL6 containing portals on B capsids as revealed by immunogold staining preparations (E). Bar in panel A = 200 nm, panel B = 20 nm. Reprinted with the authors' permission (Newcomb et al., 2001a,b).

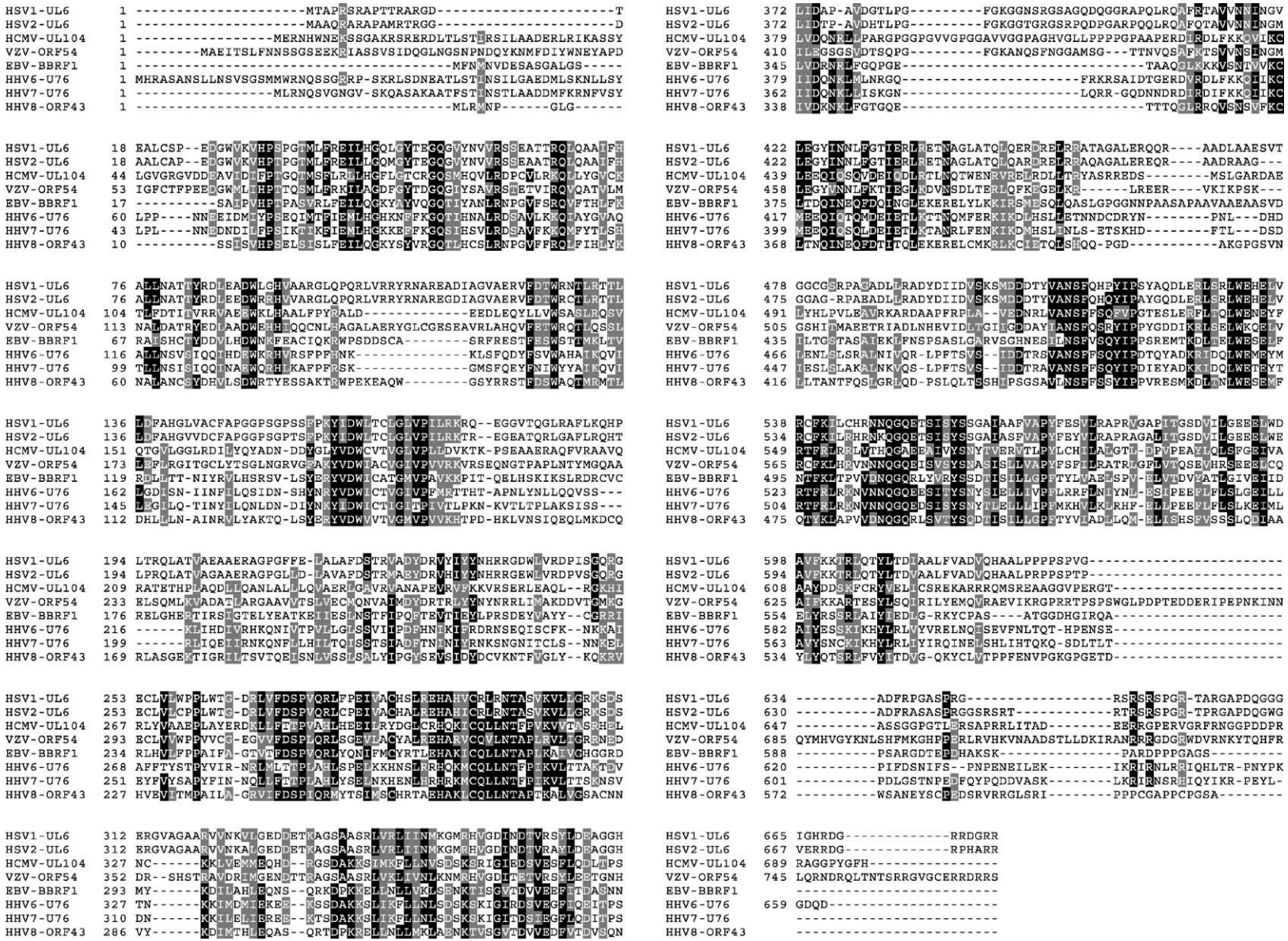

Fig. 5. Alignment of the portal protein homologues for all eight of the human pathogenic herpesviruses. Shading is indicative of sequence identity (black), conservation (grey), or divergence (white).

mutants containing loss-of-function mutations in the UL6 gene are used to infect cells, the viral progeny DNA fails to be packaged into capsids. UL6 deletion mutants are defective in both DNA cleavage and packaging and result in large numbers of empty capsids in nuclei of mutant infected cells. Electron microscopy results confirm the phenotype predicted for the inhibition of DNA packaging events, namely a lack of DNA-filled capsids in the nucleus of compound treated infected cells (Fig. 3) (van Zeijl et al., 2000; Visalli et al., 2003).

The UL6 protein has recently been shown to assemble into ring-like structures in vitro (Fig. 4A–D) that may form a portal for entry (Fig. 4E) of viral DNA into the capsid (Newcomb et al., 2001a,b; Newcomb and Brown, 2002). The VZV ORF54 protein shows 44% amino acid identity with its HSV-1 UL6 homologue (Table 2, Fig. 5), and although not yet formally proven, it is reasonable to speculate that the ORF54 protein may perform a functional role similar to that of UL6 during VZV replication. The similar EM results for infected cells treated with compounds are likely predictive of conserved functions for UL6 and ORF54. Hence, the activities of both series appear to target the same aspect of the cleavage and packaging process, and provide a new class of antiviral compounds.

8. Other potential candidates inhibiting cleavage and packaging

Whereas many antiherpes compounds with undetermined mechanisms have been discovered, only one other compound has been described in the literature that may be an inhibitor of the encapsidation process. 5-Chloro-1,3-dihydroxyacridone (Fig. 2), a cellular topoisomerase II inhibitor, was found to possess anti-HSV activity (Bastow et al., 1994; Akanitapichat et al., 2000; Akanitapichat and Bastow, 2002). This compound inhibited DNA maturation with an IC_{50} of 10 μM, but also resulted in a reduction in capsid production. The current evidence points to an indirect effect in inhibiting viral cleavage and packaging as a result of decreased numbers of packaging competent B capsids (Akanitapichat and Bastow, 2002). Drug resistant mutants have not yet been isolated and hence, further experiments will be needed to reveal the target of these acridone compounds.

9. DNA encapsidation as an antiviral target

The results presented here suggest that the encapsidation process in general, and the alphaherpesvirus portal and terminase proteins in particular (Fig. 5), are valid targets for anti-herpesvirus chemotherapy. Identification of these new classes of encapsidation inhibitors not only uncovered new valid targets for antiviral therapy, but also provide a way to analyze the role of the DNA encapsidation proteins in the cleavage and packaging process. The compounds may prove useful in helping to understand the complex protein–protein and protein–DNA interactions that occur in the nucleus during cleavage and packaging events (Fig. 1). Future studies are underway to address (i) the interaction of a compound with its respective portal protein; (ii) the interaction of portal protein with the capsid; and/or (iii) the interaction of portal protein with other cleavage and packaging proteins.

10. Conclusions

There are a number of potential herpesvirus targets amenable to antiviral chemotherapy that have not yet been fully explored. Several distinct groups of inhibitors have been identified that appear to target either (a) the terminase complex of betaherpesviruses, or (b) the portal protein of alphaherpesviruses. A review of therapies currently available for the treatment of herpesvirus infections reveals an obvious need for the development of novel therapies. Clearly, the emergence of drug-resistant virus strains is of extreme clinical significance. It is estimated that 5–25% of immunocompromised patients receiving long-term prophylactic treatment with acyclovir or ganciclovir present with resistant herpesvirus infection. Kinase deficient strains of HSV, VZV or HCMV display cross-resistance to the various nucleoside inhibitors and suppression can only be achieved with thymidine kinase-independent drugs such as foscarnet and cidofovir. The identification of compounds that target the terminase or portal proteins suggests that the DNA cleavage and packaging process is a viable target for chemotherapy of *Herpesviridae*.

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