

Mutations distal to the substrate site can affect Varicella Zoster Virus Thymidine Kinase activity: implications for drug design.

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Abbreviations:

VZVTK, varicella zoster virus thymidine kinase ;
HSV1TK, herpes simplex virus 1 thymidine kinase ;
BVDU, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine ;
IVDU, (*E*)-5-(2-iodovinyl)-2'-deoxyuridine;
BvaraU, (*E*)-5-(2-bromovinyl)-1- β -arabinosyluracil;
araT, 1- β -D-arabinofuranosylthymine;
FIAU, 2'-fluoro-2'-deoxy-beta-D: -arabinofuranosyl-5-iodouracil;
CF 1368, 3-(2'-deoxy- β -D-ribofuranosyl)-6-octyl-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one ;
CF1743,3-(2'-deoxy- β -D-ribofuranosyl)-6-(4-*n*-pentylphenyl)-2,3-dihydrofuro[2,3-*d*]pyrimidin-2-one
TMP, TDP, TTP, Thymidine mono, di, tri, phosphate;
BCNA, bicyclic nucleoside analogs;

Abstract

Varicella Zoster Virus encodes a thymidine kinase responsible for activation of antiherpetic nucleoside prodrugs such as acyclovir. Additionally, herpes virus thymidine kinases are being explored in gene/chemotherapy strategies aimed at developing novel anti-tumour therapies. In order to investigate and improve compound selectivity, we report here structure-based site-directed mutagenesis studies of VZVTK. Earlier reports showed that mutating residues at the core of the VZVTK active site invariably destroyed activity, hence we targeted more distal residues. Based on the VZVTK crystal structure, we have constructed six mutants (E59S, R84V, H97Y/A, Y21H/E), and tested substrate activity and competitive inhibition for several compound series. All VZVTK mutants tested retained significant phosphorylation activity with dThd as substrate, apart from Y21E (350-fold diminution in the k_{cat}/K_m). Some mutations give slightly improved affinities: bicyclic nucleoside analogs (BCNAs) with a p-alkyl-substituted phenyl group appear to require aromatic ring stacking interactions with residue 97 for optimal inhibitory effect. Mutation Y21E decreased the IC_{50} for the BCNA Cf1368 4-fold, whereas mutation Y21H increased the IC_{50} by more than 15-fold. These results suggest that residue 21 is important for BCNA selectivity, and might explain why HSV1TK is unable to bind BCNAs. Other mutants such as the E59S and R84V TKs, which in wild-type VZVTK stabilise the dimer interface, give opposite results regarding the level of sensitivity to BCNAs. The work described here shows that distal mutations which affect the VZVTK active-site may help in the design of more selective substrates for gene suicide therapy or as anti-varicella-zoster virus drugs.

Introduction

Varicella zoster virus is one of the eight herpes viruses that can infect humans. It is responsible for primary infections: varicella (or chickenpox) and a lifelong latent infection that reactivates periodically: zoster (or shingles) (Stevens, 1989). Varicella is generally benign but can be life-threatening especially for the immunocompromised host, or during pregnancy for the fetus (Naesens and De Clercq, 2001).

Current drug treatment of herpes virus infections is via nucleoside analogues such as acyclovir (Fig. 1) (Cohen et al., 1999; Elion et al., 1977) which act as prodrugs that undergo initial selective activation steps to 5'-diphosphates by virally encoded thymidine kinases. Further phosphorylation to 5'-triphosphates by cellular kinases yields a substrate for the viral DNA polymerase, which then can act as a competitive inhibitor and DNA chain terminator (Naesens and De Clercq, 2001). Currently, efforts are being made to improve the existing anti-varicella zoster virus treatments such as acyclovir which has relatively low potency against this virus. The class of highly potent and selective bicyclic nucleoside analogues (BCNAs) (Fig. 1) represent one example of these efforts (Balzarini and McGuigan, 2002a; Balzarini and McGuigan, 2002b; McGuigan et al., 2000; McGuigan et al., 1999). Also there are a number of studies underway aimed at using the Varicella zoster virus thymidine kinase genes for gene/chemotherapy approaches to target tumours (Degreve et al., 1997; Grignet-Debrus and Calberg-Bacq, 1997).

Herpes virus thymidine kinases are able to transfer a γ -phosphate from ATP to the 5' hydroxyl group of thymidine. Additionally VZVTK and HSV1TK have thymidylate kinase activity which converts thymidine mono-phosphate (TMP) to thymidine di-phosphate (TDP). Subsequently, TDP is converted to thymidine tri-phosphate (TTP) by cellular kinases.

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Compared to mammalian thymidine kinases, viral thymidine kinases have a broader substrate specificity and can phosphorylate many nucleoside analogs including purine derivatives. This variation in substrate specificity is the basis for the action of nucleoside analogs as antiherpes drugs and can be attributed to differences in the active sites of the viral compared to host thymidine kinases.

The interest in the potential of suicide genes and in antiherpetic drugs has encouraged studies of viral thymidine kinases and their ligands. Each thymidine kinase shows different affinities for various nucleoside analogs (De Clercq, 2004). Knowing the structure of these enzymes and their active site architecture can assist in the design of improved drugs and, in the case of gene suicide therapy, can help to direct their engineering by site-directed mutagenesis for improved or more selective thymidine kinases.

We previously reported the crystal structure of VZVTK in a product complex with BVDU-MP and ADP (Bird et al., 2003), which was used as the basis for the current mutagenesis studies. The previously reported site-directed mutagenesis of residues making direct contact with the thymidine pyrimidine ring, invariably lead to almost complete loss of enzyme activity, even when substituting HSV1TK residues into VZVTK (Roberts et al., 1991; Suzutani et al., 1993). As it was necessary to retain significant enzyme activity, we tried a more subtle approach. Analysis of the VZVTK structure suggested the mutation of some residues more distal to the active site may indirectly affect substrate binding. At the VZVTK dimer interface, a loop is stabilised by hydrogen bonds (Fig. 3). These interactions are absent in HSV1TK. The hydrogen bonded residues are E59, H88 and R84, (E59 and H88 belonging to chain A and R84 to chain B). Without such stabilising interactions, the loop conformation is thus altered in the HSV1TK structure, and it is responsible for different positioning of Y66 and Q95 in the active site. We decided to mutate these residues to their counterparts in HSV1TK *viz*: E59S and R84V, with the expectation that they could indirectly

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affect the conformation of the active site, by introducing more flexibility. Since certain molecules like ganciclovir have much higher affinity for HSV1TK than for VZVTK, we also decided to mutate residues H97 and Y21 to the corresponding HSV1TK residues, respectively Y132 and H58 (Fig 2). H97 is positioned closer to the bromo-vinyl substituent of BVDU than would be the HSV1TK homologue Y132; whereas Y21 makes weaker van der Waals contacts with the ribose sugar compared to H58.

This mutational analysis has revealed how particular residues may contribute to the binding of nucleoside analogues such as the BCNAs. Such information should be of value for the rational design of new antiherpetics and for engineering more selective thymidine kinases for use in gene therapy of tumors.

Materials and methods

Cloning

The plasmid pGEX6P1-VZVTK, described previously (Bird et al., 2003), was used to generate six mutants of VZVTK (R84V, E59S, H97Y, H97A, Y21H and Y21E) using the Quickchange mutagenesis kit from Stratagene. The presence of mutations was checked by DNA sequencing.

Expression and purification

The expression plasmid pGEX6P1-VZVTK was transformed into Rosetta(DE3)plysS cells for protein expression. Cultures were grown at 37° C in Luria Broth medium supplemented with 50 µg/ml carbenicillin and 34 µg/ml chloramphenicol. When the A_{600} reached 0.7, the cells were induced with 1mM isopropyl-beta-D-thiogalactopyranoside and incubated for an additional 16 hours at 25°C. Cells were harvested by centrifugation and resuspended in buffer A (50 mM Tris/HCl pH 7.4, 200 mM NaCl, 1mM dithiothreitol, 2% sarkosyl) + 1mM Phenylmethylsulphonylfluoride and then disrupted by sonication. The supernatant was clarified by centrifugation and applied to a 5ml glutathione-sepharose column (GST-trap Column, Amersham Biosciences), pre-equilibrated with buffer A. After the base line had returned to zero, 0.5mg of GST-3C protease in 5 ml of buffer A was added to the column which was then incubated overnight at 4° C. A 1ml glutathione-sepharose column was linked to the outlet of the 5ml column to trap any GST-VZVTK, GST or GST-3C protease which did not stay bound to the first column during elution with buffer A. The eluted VZVTK was concentrated to 2 mg/ml. The same purification procedures were used for all the mutants.

Radiochemical

The radio-labelled [CH₃-³H]dThd (specific radioactivity: 83 Ci/mmol) was obtained from Amersham Bioscience.

Compounds

The origin of the test compounds (Fig. 1) was as follows: BVDU was from Searle, UK; IVDU was synthesized by P. Herdewijn at the Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium; BVaraU was provided by H. Machida (Yamasa Shoyu Co., Choshi, Japan); araT was from Sigma Chemical Co., St. Louis, MO; acyclovir was from Wellcome Research Laboratories (now GlaxoSmithKline, UK); ganciclovir was from Roche (Brussels, Belgium); penciclovir was obtained from Hoechst (now Sanofi-Aventis, Paris, France). The BCNAs Cf1743 and Cf1368 were synthesized and provided by C. McGuigan, Cardiff, U.K.

Thymidine kinase assays

Kinase kinetics assays measuring the conversion of radiolabelled thymidine to thymidine mono-phosphate (TMP) were carried out in a final volume of 50 µl containing 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl₂, 10 mM dithiothreitol, 5 mM ATP, 10 mM NaF and 1.0 mg/ml bovine serum albumin. The enzyme and radio-labelled substrate concentrations were chosen in order to satisfy the Michaelis-Menten conditions for initial velocities. The reaction was started by the addition of enzyme to the assay mixture at 37° C, and was terminated by boiling. Aliquots of 45 µl were then spotted onto DE-81 discs (Whatman, Maidstone, England) at different times. Once dried, the discs were washed 3 times for 5 min,

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each in 1 mM HCOONH₄ whilst shaking, followed by 5 min in ethanol (70%). Finally, the filters were dried, placed in a scintillant and counted for ³H in a Beckman LS6500 liquid scintillation counter.

The inhibition of phosphorylation of dThd by the wild-type and mutated VZVTKs was assessed by determining the IC₅₀ of the test compounds. The following reaction conditions were used : the standard reaction mixture (50 µl) contained 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl₂, 10 mM dithiothreitol, 2.5 mM ATP, 10 mM NaF, 1.0 mg/ml bovine serum albumin, 1 µM [CH₃-³H]dThd (0.1 µCi) and an appropriate amount of test compound. The reaction was started by the addition of enzyme. The incubation was carried out at 37° C for 30 min; the rest of the reaction follows the kinase assay described above. The IC₅₀ was defined as the drug concentration required to inhibit thymidine phosphorylation by 50%.

Results

The purified wild type and mutant VZVTKs were compared regarding their ability to phosphorylate dThd. In order to examine the effects of the mutations on ligand binding and specificity, the K_m for each mutant against dThd and the IC_{50} for a wide range of inhibitors was determined: the pyrimidine nucleosides BVDU, IVDU, BvaraU, araT, FIAU; the purine nucleosides acyclovir, ganciclovir, penciclovir, and the BCNAs Cf1743 and Cf1368 (Fig. 1).

Thymidine kinase activity

The thymidine K_m for wild-type VZVTK used as a reference was 1.96 μM (Table 1), compared to literature K_m values of between 0.3 and 0.6 μM (Amrhein et al., 2000; Suzutani et al., 1993). Whilst our K_m value is above the average, VZVTK wild-type and mutants were purified and their activity was assayed using exactly the same protocol to allow comparison of results.

All six mutants have an increased K_m for dThd (Table 1), with the R84V thymidine kinase mutant showing the weakest apparent affinity ($9.1 \pm 1.5 \mu\text{M}$). Nevertheless, the K_m did not vary by more than 5-fold, indicating that the mutations did not dramatically change the binding mode for dThd. Concerning the V_{\max} the reference value for the wild-type is $14.8 \pm 2.0 \text{ nmol}/\mu\text{g protein/hr}$. Most of the thymidine kinase mutants apart from the Y21E thymidine kinase mutant, give a higher V_{\max} , ranging from a 3-fold increase for H97Y thymidine kinase to nearly complete loss of catalytic activity for Y21E thymidine kinase, with a V_{\max} of only $0.11 \pm 0.01 \text{ nmol}/\mu\text{g protein/hr}$. The values of k_{cat}/K_m did not change for H97A and H97Y thymidine kinase compared to the wild-type thymidine kinase (Table 1), showing that the reduction of the apparent affinity has been compensated by the increased catalytic rate. Mutations in the dimer interface of thymidine kinase, i.e. E59S and R84V resulted in

~50% reduction of enzyme efficiency (1.8- and 2.1-fold decrease, respectively). For Y21H thymidine kinase k_{cat}/K_m is even lower (2.5-fold) whilst there is a much more significant decrease of 350-fold for Y21E thymidine kinase.

Inhibitory potency of compounds against mutant VZVTKs

The purine nucleoside analog acyclovir (Cohen et al., 1999; Naesens and De Clercq, 2001) has been the main drug of choice against varicella zoster virus, even though its antiviral activity is much weaker than for herpes simplex virus 1 and 2 (Andrei et al., 2000). In our studies, acyclovir, ganciclovir and penciclovir (Fig.1) show an IC_{50} of greater than 500 μM for both the wild-type and mutant VZVTK (Table 2). The reported K_i for acyclovir against VZVTK is 830 μM (Roberts et al., 1991), whilst ganciclovir has a similar affinity for VZVTK. Thus none of the VZVTK mutants show significantly different behaviour in relation to this class of compound.

VZVTK has a preference for pyrimidine nucleoside analogs compared to purine nucleosides. For example, BVDU (De Clercq, 2004) has more than 10^3 fold superiority in potency over acyclovir (Ashida et al., 1997; Kussmann-Gerber et al., 1998; Roberts et al., 1991; Sienaert et al., 2002). In the case of the Y21E thymidine kinase mutant the IC_{50} for this class of inhibitors seems to be very similar to the wild type, whereas for Y21H thymidine kinase the IC_{50} is increased around 10-fold. Amongst other pyrimidine nucleoside analogs, araT and FIAU IC_{50} values indicate either weaker or very similar inhibitory capacity compared to the other compounds. Mutations seem to have little affect on the FIAU IC_{50} , which is at worst double that of wild-type, whereas for araT the IC_{50} varies from 2- to 8-fold.

CF1368 and CF1743 (Fig. 1) belong to the Bicyclic Pyrimidine Nucleoside Analog (BCNA) series of compounds which have previously been shown to be efficiently phosphorylated by VZVTK but not by HSV1TK (Balzarini and McGuigan, 2002a; Balzarini

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and McGuigan, 2002b). Thus, BCNAs represent a very potent and selective class of inhibitors of VZVTK. Surprisingly, the Y21E thymidine kinase mutant appears to be 5-fold more sensitive to BCNA Cf1368 than the wild-type (Table 2). In contrast, for the Y21H mutant thymidine kinase, weaker inhibition compared to wild-type for all compounds tested was observed. The BCNAs seem particularly sensitive to the mutation E59S besides Y21H. Indeed, the IC_{50} has increased 5- and 30-fold, respectively for Cf1368 and Cf1743, the highest such values observed for the E59S mutation in VZVTK.

Discussion

Effect of mutating residues at the VZVTK dimer interface

In the VZVTK subunit interface (Fig. 3), there is a change in conformation within the loop defined by residues 55 to 61 relative to HSV1TK, which makes dimer interface contacts have differences between 1.0 and 4.4 Å in α -carbon positions. The position of active site residues Y66 and Q95 following the loop are related to it. Therefore the residues E59 and R84 were mutated to their equivalents in HSV1TK, respectively serine and valine, aimed at destabilising the VZVTK loop conformation. Such mutations lost some apparent affinity for thymidine, but showed a higher V_{\max} compared to the wild-type thymidine kinase, whilst the overall enzyme catalytic efficiency (k_{cat}/K_m) is halved. These mutations show that the interactions at the dimer interface are important for the binding of thymidine, but not for the catalysis rate which has been increased. Conformational changes affecting catalytic function or inhibitor binding by mutations distal to the active site have been reported in other systems such as glucokinase and reverse transcriptase (Pedelini et al., 2005; Ren et al., 1998). These observations may be explained by the fact that the VZVTK active site residues (Y66 and Q95), following the loop could have more flexible positions, destabilising the interactions with thymidine. Consequently the apparent affinity has decreased, but the catalytic rate increased, perhaps because the greater flexibility allows easier access of the substrate to the active site.

In terms of inhibition, generally, the IC_{50} is increased by 2 to 6-fold. It is clear that the BCNAs (Fig. 1) behave differently compared to the other class of compounds. For example, the E59S mutation dramatically decreases the inhibitory capacity of Cf1743, whilst the R84V mutation increased that of Cf1368. R84 and E59 from different subunits are linked by hydrogen bonds across the dimer interface, moreover, E59 also helps to stabilise its monomer by formation of an intrasubunit hydrogen bond to H88. So we would expect a greater

destabilisation of the loop triggered by E59S than R84, which is confirmed in the case of BCNAs. In conclusion, E59S and R84V are interesting mutations in the case of BCNAs: the loop stabilisation by E59 and H88 appears essential to BCNAs, whilst the stabilisation by R84 decreases the K_i against Cf1368.

Adjusting the size of the pocket for bromovinyl substituents

The crystal structure of VZVTK (pdb code: 1OSN) shows that the 5-bromovinyl substituent of BVDU makes Van der Waals contacts in the subpocket with H97. Comparing the HSV1TK and VZVTK active sites (Fig. 2), H97 is closer to the bromine than its HSV1TK equivalent Y132. Purine nucleoside analogs are used against both varicella zoster virus and herpes simplex virus 1, but they are more potent against herpes simplex virus 1. The design of the thymidine kinase mutants H97Y and H97A was aimed to adjust the size of the subpocket around the bromine. This pocket seems smaller in the VZVTK structure compared to HSV1TK, one reason is the substitution of a tyrosine (HSV1TK) to a histidine (VZVTK). We thus decided to mutate H97Y and additionally we also made the H97A mutation in VZVTK with the opposite aim of maximising the size of the subpocket. The k_{cat}/K_m is the same as the wild type for both H97Y and H97A thymidine kinase mutants, they both have a reduced apparent affinity, and a higher V_{max} compared to the wild-type. Even if H97 does not participate in the catalysis, its mutation to a tyrosine increased the V_{max} by 3-fold. It seems that H97 plays an indirect role in the catalysis, perhaps in the correct orientation of the substrate in order to allow maximum rate enhancement.

Unfortunately, neither of the two codon 97 mutations improved the apparent affinity for bromo-vinyl substituent containing compounds. For H97A thymidine kinase, the IC_{50} has increased from 2- to 4-fold compared to the wild-type, and from 5- to 12-fold for H97Y. The mutation of residue 97 to the HSV1TK structurally homologous amino acid is worse than

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wild-type, or H97A VZVTK. For both Cf1368 and Cf1743 the same IC₅₀ is seen for the H97Y mutant compared to the wild-type thymidine kinase, whereas the K_i is increased by 6-fold for BVDU for example (Table 2). With the H97A mutant, the IC₅₀ for Cf1368 does not significantly change relative to the wild-type, but there is a 5-fold increase for Cf1743. In conclusion, adjusting the size of the subpocket did not improve the binding of bromo-vinyl substituents as expected, in fact it had rather the opposite effect, but surprisingly, the only class of compounds not affected are BCNAs whilst the IC₅₀ for all the other compounds was increased. There is one exception: H97A VZVTK against Cf1743, the only difference between Cf1743 and Cf1368 is the presence of a p-alkyl-substituted phenyl group as the side chain of the furanyl ring, instead of an alkyl side-chain (Fig. 1). We can infer that the Cf1743 phenyl group could be stabilised by aromatic ring-stacking interactions with the Y97 ring, the side chain substitution does not significantly affect Cf1368, since no interaction can be made with its aliphatic chain.

Alteration of contacts with the sugar ring

Residue Y21 was selected for mutagenesis because its equivalent in HSV1TK, H58, (Fig. 2) has a completely different hydrogen bonding pattern in the active site. Y21 makes a unique hydrogen bond to the A193 carbonyl (3 Å), whereas H58 bonds Y101 (VZVTK Y66) and with Y172 (VZVTK F139) is important for the ribose 3'-hydroxyl and pyrimidine binding, respectively. We have mutated Y21 to a histidine, the corresponding residue in HSV1TK and, additionally, mutated Y21 to a glutamic acid, which is more flexible than a tyrosine yet still able to hydrogen bond to Y66 and/or to the ribose 3'-hydroxyl. The k_{cat}/K_m is diminished for both mutants (Table 1), in the case of Y21H thymidine kinase it is decreased by almost a factor of three, whereas, Y21E thymidine kinase is 350-fold less efficient than the wild-type. This dramatic change in the catalytic efficiency is mainly due to the 100-fold

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decrease in the V_{\max} , whereas Y21H thymidine kinase shows a higher V_{\max} than the wild-type TK. It seems that the presence of a ring is important for the rate of catalysis of dThd phosphorylation, but it only has small effects on the apparent binding affinity.

Surprisingly, the IC_{50} values for all compounds against Y21E thymidine kinase are very similar to the wild-type, except for BCNAs Cf1743 and Cf1368 that show a 2.5-fold higher (Cf1743) or 5-fold lower (Cf1368) IC_{50} (Table 2). The Y21H mutation significantly decreases the affinity for BCNAs. BCNAs are known to be very selective for VZVTK and not at all able to bind HSV1TK (Balzarini et al., 2002). The exact mechanism for their antiviral activity still remains unclear, but is known to be highly dependent on phosphorylation by VZVTK (Balzarini and McGuigan, 2002a). The mutation of Y21H, which was made in order to mimic the HSV1TK active site, seems catastrophic for BCNAs. As HSV1TK is unable to bind BCNAs, it could be possible that a contributory factor for this weak affinity is the presence of a histidine in the structurally equivalent position (H58). Bird *et al.*, (Bird et al., 2003) suggested that BCNAs might adopt a significantly different binding mode in VZVTK compared with conventional nucleosides because they could not be easily modelled into the VZVTK active site based on overlaps with the BVDU deoxyribose or base positions. The Y21H mutation may force the BCNAs sugar position into a certain conformation, which is not adequate for the reaction to occur.

In conclusion, this work has provided interesting clues as to substrate specificity for TKs especially relating to BCNAs. Our study highlights a few points: the stabilisation of the dimer interface is not essential for Cf1368 binding, but for both BCNA molecules studied the integrity of the loop is important. Secondly, the BCNAs with a p-alkyl-substituted phenyl group apparently require aromatic ring stacking interactions with residue 97 for optimal inhibitory effect. Finally, a charged residue at position 21 with a long and flexible side chain improves the binding of BCNA Cf1368.

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Footnotes

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Legends for Figures

Fig. 1. Structural formulae of nucleoside analogs.

Fig. 2. Active sites superimposition of VZVTK (colored in blue) and HSV1TK (colored in grey), highlighting mutated residues.

Fig 3. Diagram showing the conformational difference at the dimer interface between VZVTK (blue) and HSV1TK (grey).

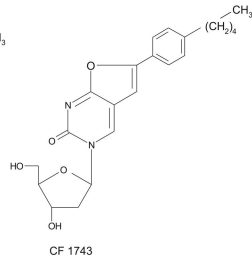
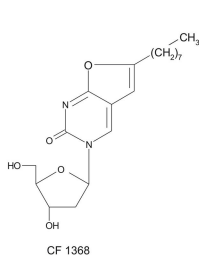
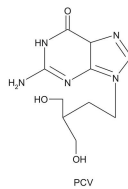
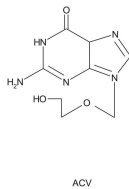
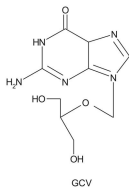
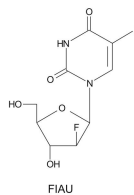
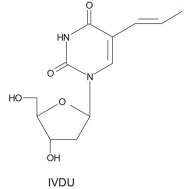
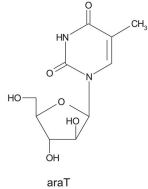
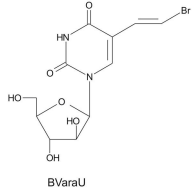
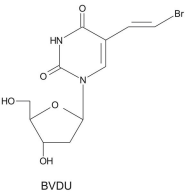
Tables

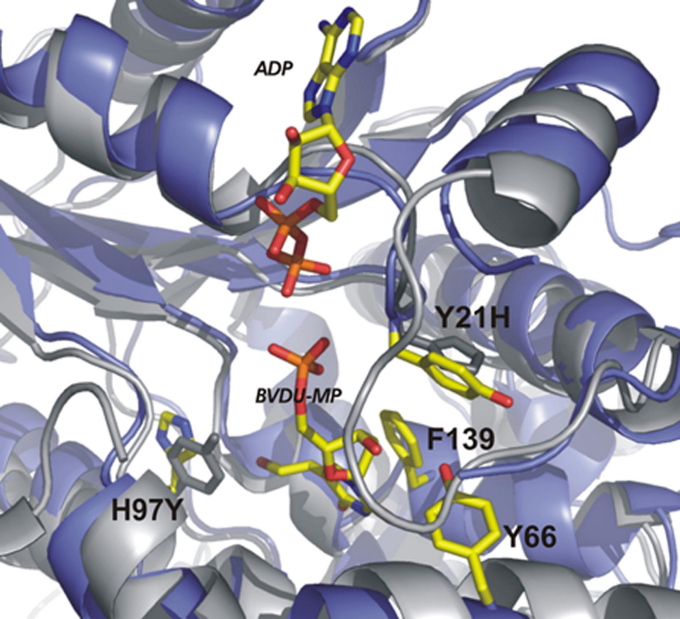
Table 1: Kinetic parameters for mutant VZVTKs catalysing the ATP-dependent phosphorylation of dThd.

| VZVTK Mutant | K_m (μM) | V_{max} (nmol/mg protein/hr) | k_{cat}/K_m ($\text{min}^{-1}/\mu\text{M}$) |
|--------------|----------------------------|--|---|
| WT | 1.96 ± 0.83 | 14.8 ± 2.0 | 17.11 |
| E59S | 5.28 ± 0.93 | 21.6 ± 1.0 | 9.27 |
| R84V | 9.14 ± 1.49 | 32.1 ± 7.1 | 7.96 |
| H97A | 3.44 ± 1.75 | 23.8 ± 0.2 | 15.68 |
| H97Y | 6.30 ± 0.63 | 43.2 ± 1.6 | 15.54 |
| Y21H | 7.67 ± 0.49 | 18.4 ± 0.3 | 5.43 |
| Y21E | 5.36 ± 4.05 | 0.11 ± 0.01 | 0.046 |

Table 2: Inhibitory potency (IC₅₀ in μM) of test compounds for mutant VZV TK-catalysed phosphorylation of dThd by ATP.

| Compounds | Mutations in VZV TK | | | | | | |
|--------------------|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| | WT | E59S | R84V | H97A | H97Y | Y21H | Y21E |
| BVDU | 1.5 ± 0.1 | 5.9 ± 0.6 | 7.0 ± 0.1 | 4.7 ± 0.3 | 10 ± 5 | 14 ± 0 | 2.2 ± 0.9 |
| IVDU | 1.2 ± 0.1 | 5.6 ± 0.0 | 5.0 ± 0.1 | 5.1 ± 0.1 | 7.3 ± 0.1 | 8.8 ± 3.1 | 1.8 ± 0.2 |
| BvaraU | 1.4 ± 0.2 | 5.2 ± 0.3 | 6.6 ± 1.9 | 3.0 ± 1.3 | 19 ± 8 | 22 ± 1 | 1.7 ± 0.2 |
| araT | 20 ± 1 | 39 ± 1 | 116 ± 55 | 80 ± 10 | 11 ± 18 | 173 ± 38 | 34 ± 2 |
| FIAU | 17 ± 1 | 35 ± 1 | 20 ± 11 | 34 ± 8 | 31 ± 3 | 34 ± 0 | 2.3 ± 0.1 |
| Acyclovir | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| Ganciclovir | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| Penciclovir | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| Cf1743 | 5.5 ± 3.3 | 298 ± 81 | 11 ± 7 | 27 ± 4 | 4.5 ± 0.5 | 223 ± 222 | 13 ± 5 |
| Cf1368 | 33 ± 1 | 161 ± 131 | 19 ± 7 | 28 ± 4 | 38 ± 1 | >500 | 7.5 ± 5.0 |





ADP

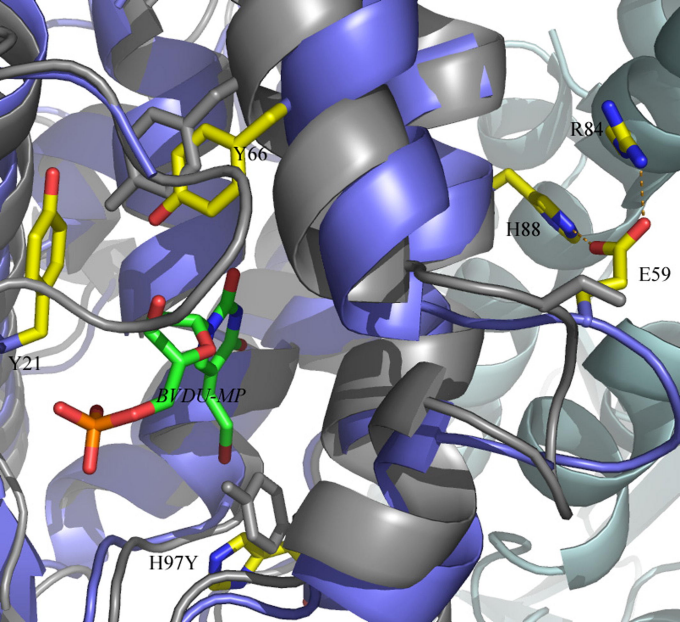
Y21H

BVDU-MP

F139

H97Y

Y66



Y66

R84

H88

E59

Y21

BVDU-MP

H97Y