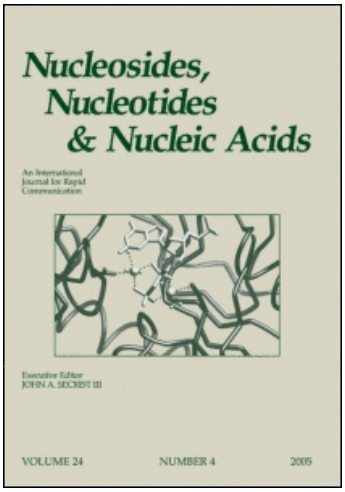

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Molecular Modeling of the Binding of 5-Substituted 2'-Deoxyuridine Substrates to Thymidine Kinase of Herpes Simplex Virus Type-1

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Molecular Modeling of the Binding of 5-Substituted 2′-Deoxyuridine Substrates to Thymidine Kinase of Herpes Simplex Virus Type-1

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ABSTRACT

In an earlier study, De Winter and Herdewijn (J. Med. Chem. **1996**, *37*, 4727–4737) studied the binding of various 5-substituted 2′-deoxyuridine substrates to thymidine kinase of herpes simplex virus type-1. They used a computational procedure that achieves good correlation with experimentally determined IC_{50} values. We applied an alternative procedure to the same deoxyuridine substrates, using only three readily calculated quantities—the binding energy, the molecular surface area, and a flexibility factor. Our simplified method achieves the same degree of correlation with the IC_{50} values as did the earlier procedure. We then applied this procedure to examine the binding of various 5-substituted pyrimidine 1,5-anhydrohexitol substrates to thymidine kinase.

Key Words: Herpes simplex virus type-1; Thymidine kinase; Herpesviridae; Deoxyuridine substrates; IC_{50} values.

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INTRODUCTION

Herpesviridae represents a family of viruses of considerable medical importance. These viruses are involved in cold sores, shingles, skin eruptions and rashes, a venereal disease, mononucleosis, brain and eye infections, birth defects, and probably several cancers.[1] The specific virus of this family responsible for the common cause of cold sores is herpes simplex virus type-1 (HSV-1).

HSV-1 codes for a specific thymidine kinase (TK) that the virus utilizes to salvage deoxythymidine for use in the growing viral genome of infected cells. This kinase has drawn much attention as a pharmaceutical target due to it ability to bind to a broad range of substrates compared to its human cellular counterpart.[2] For example, many 5-substituted pyrimidine 1,5-anhydrohexitols and 5-substituted 2′-deoxyuridine substrates have been synthesized to target HSV-1. The viral TK phosphorylates these substrates, thus activating them for subsequent use by the viral polymerase.[3]

Because of the availability of the atomic coordinates[4] from the x-ray crystallography of TK and the availability of the IC_{50} values of the synthetic substrates, researchers have applied molecular modeling to study TK and the binding of anti-HSV-1 agents.[2,3,5–8] In one of these studies, De Winter and Herdewijn[2] investigated the binding of 5-substituted 2′-deoxyuridine substrates to TK. Table 1 shows these substrates along with their corresponding IC_{50} values. They generated multiple-linear-regression plots that correlated the experimentally determined IC_{50} values of the various substrates with calculated interaction energies and free energies of solvation. These researchers generated plots of the logarithm of the IC_{50} values as a function of the calculated interaction energies, corrected for differences in hydrophobicity of the substrates. Their model produced correlation coefficients (R^2) that ranged from 0.3 to 0.8, depending on the details of how they calculated the interaction energy.

We present an alternative model that produces the same degree of correlation (correlation coefficient 0.81). Our model includes three readily derived terms: binding energy, surface area (which partially takes into account solvent effects), and rigidity (which describes the flexibility of each ligand to facilitate binding within the active site). We also apply our methodology to investigate the binding of the anhydrohexitol substrates to TK.

METHODS

The binding energy for a reversible inhibitor can be calculated as described previously.[9] The binding energy (ΔE_B) is defined as $\Delta E_B = E_{Ligand + Enzyme} - (E_{Ligand} + E_{Enzyme})$, where $E_{Ligand + Enzyme}$ is the total energy of a minimized complex, E_{Ligand} is the calculated Boltzmann average energy evaluated at 25°C of the ligand minima, and E_{Enzyme} is the energy of the minimized enzyme with no ligand in the active site. We determined E_{Ligand} using a conformational searching procedure for nucleotides and nucleosides[10] that produces an ensemble of minima from which we calculated a Boltzmann average energy.

We used molecular modeling software from Accelrys Inc. and Hypercube Inc., and performed energy minimizations using the CFF91 force field from Accelrys on a Silicon Graphics O2 workstation. To determine the surface area of each ligand, we used the HyperChem program on a Windows-based personal computer.

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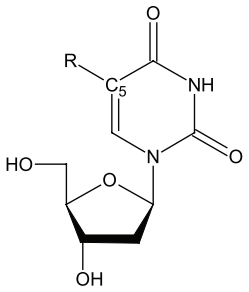
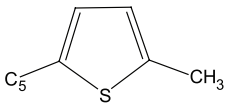
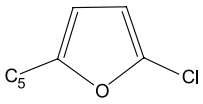
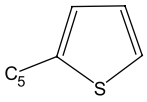
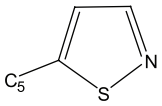
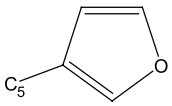
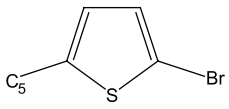
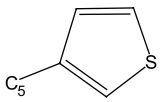
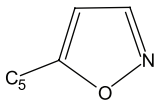
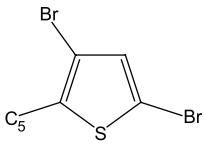
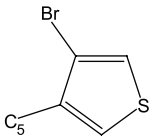
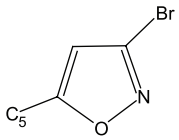
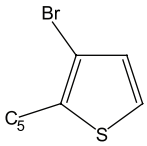
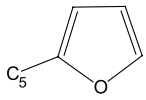
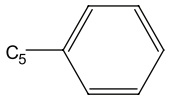
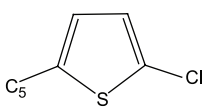
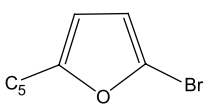
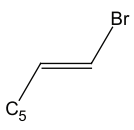
Table 1. 5-Substituted 2′-deoxyuridine substrates analyzed in this study with corresponding IC_{50} values.



	−R	IC_{50} (μM)		−R	IC_{50} (μM)		−R	IC_{50} (μM)
T	−Me	1.0	6		2.3	12		28
1		2.4	7		3.1	13		1.5
2		3.5	8		4.0	14		36
3		62	9		143	15		34
4		61	10		2.9	16		102
5		2.3	11		51	17		0.3

(Redrawn from Figure 3 of *J. Med. Chem.* **1996**, *37*, 4727–4737.)

Partial atomic charges of each molecule were assigned automatically by Insight II based on the CFF91 force field. Energy minimization of each free ligand utilized the Newton Raphson method, whereas the other energy minimizations utilized the conjugate gradient method. The degree of convergence was set for an RMS value of less than 0.01 kcal/mol-Å. All minimizations were performed without a distance cutoff in calculating the non-bonding interaction. Solvent was not included explicitly, but the dielectric constant was set to 4.0, with distance dependence.

We obtained the x-ray crystal structure of TK from the protein data bank (2VTK.pdb), which was co-crystallized with ADP, deoxythymidine, and 28 water molecules. The residues 1–45, 150–152, and 265–279 were missing due to insufficient electron density. Therefore, with Insight II, we added an acetyl group on the N terminus of residues 46, 153, 280, respectively, and a methylamine group on the C terminus of residues 149 and 264. All hydrogen atoms were assigned by Insight II at pH = 7. We optimized the newly added atoms (acetyl groups, methylamine groups, and hydrogen atoms) by minimization with all of the other heavy atoms in the crystal structure fixed. This deoxythymidine–kinase complex served as our initial structure for further minimization when we switched to the various other ligands.

For energy minimization of each enzyme–ligand complex, we employed a two-step minimization protocol. First, we restrained the entire complex, with the exception of the ligand, and minimized the energy of the ligand structure within the enzyme active site. We then performed a second minimization, allowing all atoms in the system (including enzyme residues, ligand, and solvent) within an 8 Å sphere around the ligand to vary. The docking of other ligands was facilitated by replacing the methyl group of the thymidine with proper substituents and re-performing the two-step minimization.

To assess a possible alternative binding mode of each ligand within the active site of the enzyme, the furanose sugar ring was twisted from the 2′-endo orientation to 3′-endo and the resulting complex was again subjected to the two-step minimization.

We also included a ligand flexibility term that accounts for the relative ease of deoxynucleoside binding to the enzyme active-site pocket. To determine the flexibility term in our analysis, we assigned a value of 1.0 for each double bond present in the 5-substituent. Thus, thymidine, with no double bonds, received a value of 0, compound 17 with one double bond received a value of 1, and so forth.

We used HyperChem to calculate the surface areas (SA) of each ligand, based on its global minimum. For the multiple linear regression analysis, we used the Origin™ software.

RESULTS AND DISCUSSION

Table 2 shows the computed data for each of the ligands, including the binding energy ΔE_B, the ligand surface area (SA), and the ligand rigidity (R), for thymidine and the 17 selected substrates. The number for each ligand corresponds to the previously published numbers for this set of compounds.[2] The multiple linear regression model developed from the data in Table 2 is shown in Figure 1. The least-squares equation for the linear regression is

$$\Delta E_B = 0.4256[\ln(IC_{50})] + 0.1321(SA) - 1.7823(R) - 101.4796$$

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Table 2. 5-Substituted 2′-deoxyuridine substrates analyzed in this study with corresponding ΔE_B, solvent accessible surface area, and rigidity values.

Substrate number	IC_{50} (μM)	ΔE_B (kcal/mol)	Surface area (Å^2)	Rigidity
Thy	1.0	− 42.97	441.28	0
1	2.4	− 37.05	510.29	2
2	3.5	− 33.58	540.72	2
3	62	− 30.02	561.98	2
4	61	− 30.47	538.93	2
5	2.3	− 35.80	537.97	2
6	2.3	− 32.26	542.96	2
7	3.1	− 37.38	505.23	2
8	4.0	− 35.49	512.44	2
9	143	− 28.76	535.92	2
10	2.9	− 38.35	501.90	2
11	51	− 31.08	530.88	2
12	28	− 33.13	530.27	2
13	1.5	− 39.65	493.69	2
14	36	− 40.94	493.36	2
15	34	− 38.85	522.46	2
16	102	− 32.57	531.87	3
17	0.3	− 37.87	497.53	1

The correlation R^2 is 0.81; this compared favorably with the value $R^2 = 0.8$ reported by De Winter and Herdewijn.[2] The principal difference between their model and ours is the following: we calculated binding energies rather than interaction energies. The binding energies are more complete because they take into account not only the interactions between the ligand and enzyme but also the conformational energy change in the enzyme and the ligand upon binding. Others[11,12] likewise have computed binding energies similar to ours in studying enzyme–ligand binding.

Neither the calculated binding energy ΔE_B nor interaction energy includes all the factors that go into the experimentally observed IC_{50} values. Other factors include poor solubility of the ligand, a poor initial interaction with the enzyme in the unbound

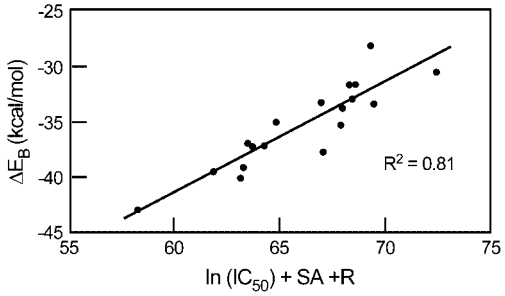

Figure 1. A plot of $\ln(IC_{50}) + SA + R$ vs. binding energy (ΔE_B).

state, the inability to undergo the necessary conformational changes for binding, enzyme desolvation, and differences in entropy changes. These factors are difficult to determine accurately,[2,11] but some are roughly approximated by the molecular surface area and by the flexibility factor, as we have tried to do. Obviously, if we were able to take all factors into account properly, the correlation coefficient in Figure 1 would approach unity.

De Winter and Herdewijn[2] observed that the interaction energy between substrate and enzyme decreases as the size of the thienyl or furanyl substituent increases. This implies that the binding might depend not only on the binding energy but also on the surface area of ligand. To support this idea, Tossi et al.[11] determined that $P_2/P_{2'}$-substituent bulkiness of HIV aspartic protease inhibitors was an important factor in binding and included a surface area term to represent the bulkiness of the $P_2/P_{2'}$-substituent in their regression analysis. We therefore decided to include a surface area term (SA) to partially account for the differences in enzyme affinity for the various 5-substituted ligands of HSV-1 thymidine kinase.

Tossi et al.[11] also concluded that decreased binding capability could be correlated to bulky conjugated rings in the $P_2/P_{2'}$-substituents of the protease inhibitors. They attributed this to delocalization onto the aromatic ring system of the substituents, which reduced the torsional flexibility of the ligand and thereby decreased the ability of the ligand to form hydrogen bonds with the enzyme. Thus, Tossi et al.[11] also included a flexibility term in their analysis to account for torsional constraints.

Therefore, we also chose to include a flexibility term, because most of the HSV-1 thymidine kinase substrates listed in Table 1 possess conjugated ring substituents at the 5-position of the pyrimidine base, suggesting that many of these substrates might also have reduced ability to fit into the active site of the target enzyme. In fact, our analysis showed that most of these substrates have restricted flexibility. Specifically, most of the substrates have only two orientations of the substituent ring, both of which were approximately co-planar with the pyrimidine ring, meaning that they have restricted conformational freedom, which decreases the rate at which they can bind to the active site of TK.

The importance of including the flexibility term is also suggested by the shape of the TK active site. For example, Alber et al.[5] concluded that Met 128 in TK sterically limits the orientation of the 5-substituent in the substrate, and De Winter and Herdewijn[2] indicated that Tyr 132 must be slightly displaced to allow binding of the 5-substituent groups. Perhaps these residues account for an initial unfavorable interaction between the enzyme and the bulky 5-substituent of the substrates. The more flexible the substrate, the more readily it can overcome the steric interactions with Met 128 and Tyr 132.

Analysis of the regression plot indicates significant correlation ($R^2 = 0.81$) between experimentally determined IC_{50} values and our calculated variables of ΔE_B, solvent accessible surface area, and ligand flexibility. When we omitted the ligand flexibility term, the correlation was still 0.76, in agreement with the value of 0.8 obtained by the De Winter and Herdewijn method. The decrease in R^2 by only 0.05 upon omitting the flexibility term suggests that flexibility plays a small role in predicting IC_{50} values.

We observe a much higher correlation coefficient (0.8) for our model with its corresponding 8-Å distance cut off limit than did the De Winter and Herdewijn (only

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0.2) with the same distance cutoff. This improved correlation suggests that using the binding energy ΔE_B rather than the interaction energy gives more accurate results at the 8-Å distance cut off limit.

We now turn our attention to the predictive power of the regression model. We attempt to predict IC_{50} values from the calculated ΔE_B, surface area, and flexibility values by moving the variables to different axes of the linear regression plot. When we make IC_{50} the dependent variable (plotted on the Y axis) and make ΔE_B + surface area + flexibility the independent variable, we obtain a poor correlation coefficient of 0.48. A similar result is obtained using the De Winter and Herdewijn data. This indicates that neither our method nor that of De Winter and Herdewijn can be used to predict IC_{50} values accurately, although both methods might be useful in designing substrates and inhibitors of TK.

To further illustrate the difficulty of using our model for predictive purposes, we divide the test compounds into two groups, namely a training set on which a QSAR model is built and a test set that can be used to prove predictive power.

Compounds 9 through 17 serve as our training set so as to include the compounds with the lowest and highest experimentally determined IC_{50} values. The least-squares equation for this linear regression is

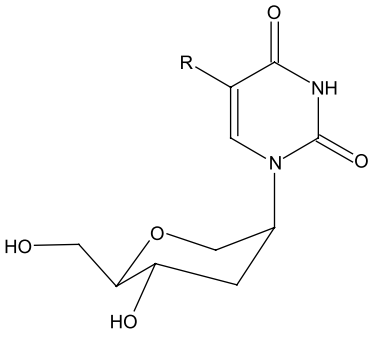
$$\ln(IC_{50}) = 0.05047(\Delta E_B) + 0.05393(SA) + 1.89148(R) - 27.08095$$

which yields a correlation R^2 value of 0.73 for this training set model.

Deoxythymidine and compounds 1 through 8 serve as our test set. We used the training set model described above to predict $\ln(IC_{50})$ values for the test set compounds and the results are plotted against the experimentally determined $\ln(IC_{50})$ values. If the training set model has predictive power, the correlation coefficient should approach unity. However, the correlation R^2 is only 0.35, indicating again that the model lacks sufficient predictive power.

We now focus on our analysis of the binding of the anhydrohexitol substrates to TK. Table 3 shows the ΔE_B values for the axial and equatorial geometries of the

Table 3. Binding energies ΔE_B of 5-substituted pyrimidine 1,5-anhydrohexitol substrates to thymidine kinase of HSV-1 (axial conformation is shown).



R	ΔE_B (kcal/mol) Equatorial conformation	Axial conformation
I	− 39.9	− 34.7
CH_2CH_3	− 42.5	− 40.0
CF_3	− 47.5	− 42.3
Cl	− 43.6	− 38.4
CH_3	− 42.2	− 36.9

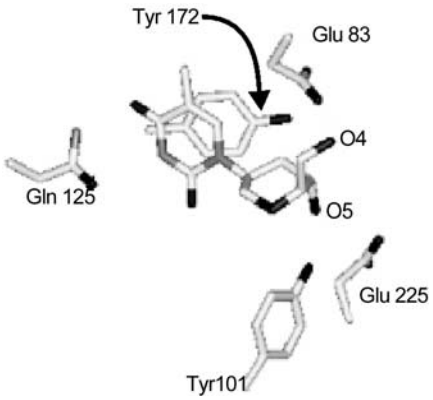


Figure 2. Minimized structure of the equatorial geometry of the R = CH_3 hexitol substrate in complex with HSV-1 TK.

six-membered ring of the anhydrohexitol substrates in the active site of HSV-1 thymidine kinase. In all cases we observe that the ΔE_B for the equatorial geometry (more negative) is favored over the ΔE_B of the axial geometry, in agreement with the observed equatorial geometry of iodo-hexitol nucleoside when bound to HSV-1 thymidine kinase.[13]

A comparison of the total potential energies (data not shown) of both geometries of the R = CH_3 hexitol substrate bound to HSV-1 thymidine kinase indicates a 10.71 kcal/mol energy difference. A corresponding total potential energy difference of 10.25 kcal/mol was previously observed using the AMBER force field.[3] Figure 2 shows the minimized conformation of the equatorial geometry of this molecule in the active site of HSV-1 thymidine kinase, which is similar to that obtained in the previous study.[3] The general conformational characteristics including hydrogen bonding with critical enzyme residues are preserved.

The trifluoromethyl hexitol nucleoside is the most potent of this series of the 5-anhydrohexitol substrates. Of the studied hexitol nucleosides, the trifluoromethyl hexitol was found to have the smallest ΔE of conversion between the equatorial and the axial geometries.[3] The unbound substrate conformation maintains the axial geometry but converts to the equatorial geometry when bound in the active site of TK. Wouters and Herdewijn concluded that further investigation of the relationship between ΔE and biological activity might show why the trifluoromethyl hexitol nucleoside is the most potent.

In our study, we have found that the trifluoromethyl hexitol nucleoside has the most negative ΔE_B value (-47 kcal/mol) of this series of substrates, indicating that it binds most favorably to TK in comparison to the other hexitol substrates. We hypothesized that the explanation for this low binding energy might the favorable electrostatic interactions between the high electron density of the electronegative fluorines and possible positively charged residues of the TK enzyme. This hypothesis, however, proved incorrect. The favorable binding energy of the trifluoromethyl hexitol

nucleotide is due to many, small electrostatic and nonbonded interaction energies, and not due to one or even several strong electrostatic interactions.

We also observe that the ΔE_B value for the binding of the trifluoromethyl hexitol substrate to TK is more negative than for the natural deoxythymidine substrate. On the other hand, a recent investigation[6] showed that the k_{cat} value for deoxythymidine is higher than for the trifluoromethyl substrate, indicating that the magnitude of ΔE_B may not always be directly related to k_{cat}.

Finally, we compared the geometries of our calculated binding complexes to those of the x-ray structures.[14] For each of the three reported x-ray structures,[14] we focused on the drug active-site residues 83, 101, 125, 172, and 225 and the bound drug, quantitatively comparing this part of the complex in our computed lowest-energy structure with the corresponding part of the x-ray crystal structures. When compound **2** (see Table 1) is bound to thymidine kinase as reported in the PDB structure 1KI4, the conformation of **2** is the 2′-endo rather than the 3′-endo conformation. Our calculated structure was also lower in energy in the 2′ than the 3′-endo conformation. Furthermore, in comparing residues (those around the active site) along with the bound drug, our calculations differed from x-ray crystal structure by an RMS of only 0.46 Å (molecule 1 of the dimmer) and 1.23 Å (molecule 2 of the dimmer). Similarly, our calculated structure of the designated part of the complex of compound **17** with thymidine kinase differed from the reported[14] x-ray structure 1KI8 by an RMS of 0.90 Å (molecule 1) and 0.93 Å (molecule 2), with both the calculated and the x-ray structures showing compound **2** in the 2′-endo conformation. Finally, our calculated structure of the bound compound **21** appeared in the 1C conformation, as did bound **21** in the 1KI6 x-ray structure,[14] even though the C1 conformation was favored over 1C in the unbound compound **21**. The RMS deviation between the designated part of our calculated complex of **21** with thymidine kinase differed by only 0.53 Å (molecule 1) and 0.79 Å (molecule 2). These data indicate that our calculated structures are close to those of the x-ray crystal structures and support the validity of our binding-energy calculations.

CONCLUSIONS

We conclude that the inclusion of three readily derived terms of ΔE_B-solvent accessible surface area, and ligand flexibility—can produce a high correlation with the experimentally determined IC_{50} values. Although our method and other similar computational methods might be useful in designing substrates and inhibitors of TK, they might not be useful in accurately predicting IC_{50} values per se. Therefore, future efforts will be directed towards improving predictive capability. Our methodology may also be applied to other less experimentally studied ligands (such as the anhydrohexitol substrates) to gain an understanding of important factors related to TK binding.

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REFERENCES

1. Levy, J.A.; Fraenkel-Conrat, H.; Owens, R.A. Viruses with medium and large DNA genomes. In *Virology,* 3rd Ed.; Brake, D.K., Liguori, B., Eds.; Prentice-Hall, Inc.: New Jersey, 1994; 192–198.
2. De Winter, H.; Herdewijn, P. Understanding the binding of 5-substituted 2′-deoxyuridine substrates to thymidine kinase of herpes simplex virus type-1. J. Med. Chem. **1996**, *39*, 4727–4737.
3. Wouters, J.; Herdewijn, P. 5-Substituted pyrimidine 1,5-anhydrohexitols: conformational analysis and interaction with viral thymidine kinase. Bioorg. Med. Chem. Lett. **1999**, *9*, 1563–1566.
4. Vogt, J.; Perozzo, R.; Pautsch, A.; Prota, A.; Schelling, P.; Pilger, B.; Folkers, G.; Scapozza, L.; Schulz, G.E. Nucleoside binding site of herpes simplex type 1 thymidine kinase analyzed by x-ray crystallography. Proteins **2000**, *41*, 545–553.
5. Alber, F.; Kuonen, O.; Scapozza, L.; Folkers, G.; Carloni, P. Density functional studies on herpes simplex virus type 1 thymidine kinase–substrate interactions: the role of Tyr-172 and Met-128 in thymine fixation. Proteins **1998**, *31*, 453–459.
6. Sulpizi, M.; Schelling, P.; Folkers, G.; Carloni, P.; Scapozza, L. The rational of catalytic activity of herpes simplex virus thymidine kinase. J. Biol. Chem. **2001**, *276* (24), 21692–21697.
7. Wang, J.; Froeyen, M.; Hendrix, C.; Andrei, G.; Snoeck, R.; De Clercq, E.; Herdewijn, P. The cyclohexene ring system as a furanose mimic: synthesis and antiviral activity of both enantiomers of cyclohexenylguanine. J. Med. Chem. **2000**, *43*, 736–745.
8. Kussmann-Gerber, S.; Kuonen, O.; Folkers, G.; Pilger, B.D.; Scapozza, L. Drug resistance of herpes simplex virus type 1 structural considerations at the molecular level of the thymidine kinase. Eur. J. Biochem. **1998**, *255*, 472–481.
9. Nair, A.C.; Miertus, S.; Tossi, A.; Romeo, D. A computational study of the resistance of HIV-1 aspartic protease to the inhibitors ABT-538 and VX-478 and design of new analogues. Biochem. Biophys. Res. Commun. **1998**, *242*, 545–551.
10. Harris, D.G.; Shao, J.; Anderson, J.M.; Marx, D.P.; Zimmerman, S.S., *in press*.
11. Tossi, A.; Bonin, I.; Antcheva, N.; Norbedo, S.; Benedetti, F.; Miertus, S.; Nair, A.C.; Maliar, T.; Dal Bello, F.; Palu, G.; Romeo, D. Aspartic protease inhibitors: an integrated approach for the design and synthesis of diaminodiol-based peptidomimetics. Eur. J. Biochem. **2000**, *267*, 1715–1722.
12. Jayatilleke, P.R.N.; Nair, A.C.; Zauhar, R.; Welsh, W.J. Computational studies on HIV-1 protease inhibitors: influence of calculated inhibitor-enzyme binding affinities on the statistical quality of 3D-QSAR CoMFA models. J. Med. Chem. **2001**, *43*, 4446–4451.
13. Ostrowski, T.; Wroblowski, B.; Busson, R.; Rozenski, J.; De Clercq, E.; Bennett, M.S.; Champness, J.N.; Summers, W.C.; Sanderson, M.R.; Herdewijn, P. 5-Substituted pyrimidines with a 1,5-anhydro-2,3-dideoxy-D-arabino-hexitol moiety at N-1: synthesis, antiviral activity, conformational analysis, and interaction with viral thymidine kinase. J. Med. Chem. **1998**, *41*, 4343–4353.

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14. Champness, J.N.; Bennett, M.S.; Wien, F.; Visse, R.; Summers, W.C.; Herdewijn, P.; de Clerq, E.; Ostrowski, T.; Jarvest, R.L.; Sanderson, M.R. Exploring the active site of herpes simplex virus type-1 thymidine kinase by x-ray crystallography of complexes with aciclovir and other ligands. Proteins **1998**, *32*, 350–361.

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