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Prediction of the binding mode of N^2-phenylguanine derivative inhibitors to herpes simplex virus type 1 thymidine kinase

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Summary

The probable binding mode of the herpes simplex virus thymidine kinase (HSV1 TK) N^2-[substituted]-phenylguanine inhibitors is proposed. A computational experiment was designed to check some qualitative binding parameters and to calculate the interaction binding energies of alternative binding modes of N^2-phenylguanines. The known binding modes of the HSV1 TK natural substrate deoxythymidine and one of its competitive inhibitors ganciclovir were used as templates. Both the qualitative and quantitative parts of the computational experiment indicated that the N^2-phenylguanine derivatives bind to the HSV1 TK active site in the deoxythymidine-like binding mode. An experimental observation that N^2-phenylguanosine derivatives are not phosphorylated during the interaction with the HSV1 TK gives support to the proposed binding mode.

Introduction

There are seven herpes viruses that use humans as host: herpes simplex virus type 1 (HSV1), herpes simplex virus type 2 (HSV2), varicella-zoster virus, cytomegalovirus, human herpes virus of types 6 and 7 and Epstein-Barr virus [1]. HSV1 is most common to affect the upper parts of the body, especially the mouth (recurrent herpes labialis and gengivostomatitis), the eyes (keratoconjunctivitis) and the brain (meningitis and encephalitis). HSV2 is usually transmitted sexually and is the cause of genital ulcerations (primary and recurrent genital herpes) and the generalised neonatal infection, transmitted by the infected mother during childbirth. The combating of HSV infections is normally based on the inhibition of the viral thymidine kinase (TK), which is an essential enzyme as it is responsible for the monophosphorylation of deoxythymidine, whose triphosphorylated form is the raw material for viral DNA synthesis. HSV TK has been proved to be a good target for anti-HSV drugs because of its low similarity to human TK (approximately 21%, considering total identity, and 42% considering functional homology; see experimental section for details) and its unusual capacity to phosphorylate a wide range of nucleoside-like molecules. In medical practice, acyclovir is the drug of choice to be used against herpes infections, but many other categories of HSV TK inhibitors have also been described [2].

Wright et al. [3] described a set of N^2-phenylguanine derivatives (Figure 1) as potential selective anti-herpes agents. Although this class of compounds is not currently in use in medical practice, the results of the qualitative [4] and quantitative [5] studies performed by Wright et al. shed some light on the mechanism of HSV1 TK-inhibitor interaction.

Since the publication of the crystal structure of HSV1 TK in complex with deoxythymidine and ganciclovir [6, 7] (see a model of the crystal structure of HSV1 TK in complex with deoxythymidine in Figure 2 and the structures of deoxythymidine and gan-

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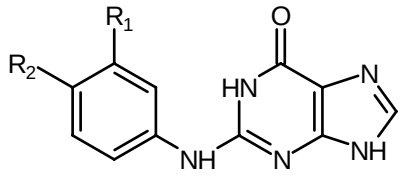

Figure 1. N^2-Phenylguanine derivatives.

ciclovir in Figure 3), it has become possible to carry out more accurate investigations about the mechanism of HSV TK–ligand interaction. Important information provided by the crystal structure is the binding mode of the HSV1 TK natural substrate deoxythymidine, and one of its competitive inhibitors, ganciclovir. In the present work, the binding modes of deoxythymidine and ganciclovir are used as templates in a computational investigation to find out the most probable binding mode of N^2-phenylguanines.

Binding modes of deoxythymidine and ganciclovir

The binding modes of deoxythymidine and ganciclovir are sketched in Figure 4. Both molecules bind in almost the same way to the HSV1 TK active site. The most important HSV1 TK-ligand interactions are: (a) the formation of a double hydrogen bond between the purine/pyrimidine ring of the ligand and Gln 125, which works as an anchor to the ligand molecule; (b) the spatial orientation of the benzene ring of Tyr 172, parallel to the ligand purine/pyrimidine ring, and the SCH_3 group of Met 128, pointing to the middle of the ligand purine/pyrimidine ring, serving as additional support to stabilise the ligand position; (c) the 3′-OH forms a hydrogen bond with Tyr 101; and (d) 5′-OH is kept in the correct position to be phosphorylated through the formation of another hydrogen bond with Arg 163. Although very similar, the binding modes of deoxythymidine and ganciclovir present a small, but important difference. The double hydrogen bonds formed between the two ligands and Gln 125 are not exactly the same; the amide group of Gln 125 is rotated by 180°.

Possible binding modes of N^2-phenylguanines

In order to find out the correct binding mode of N^2-phenylguanines, we started from the assumption that their guanine ring interacts with Gln 125, Tyr 172 and Met 128 in either deoxythymidine-like or ganciclovir-like binding mode. This is a reasonable starting point, considering the similarity in the binding modes of deoxythymidine and ganciclovir. Figure 5 shows the ganciclovir-like binding mode of N^2-phenylguanines. In this binding mode, the position of the guanine ring was adjusted to match the guanine ring of the ganciclovir molecule (Figure 5a). In doing so, there are two possible orientations for the phenyl ring; one in which the torsion angle ϕ_1 is approximately 0° (Figure 5b) and another in which ϕ_1 is approximately 180° (Figure 5c). Values too much different from 0° and 180° are forbidden due to steric overlaps between the phenyl ring and neighbouring amino acid residues. The deoxythymidine-like binding mode of N^2-phenylguanines is shown in Figure 6. The position of the guanine ring was adjusted to match the double hydrogen bond of deoxythymidine (Figure 6a). However, when the guanine ring is in this configuration only one phenyl ring conformation is possible, that is, the one in which the torsion angle ϕ_1 is approximately 180° (Figure 6b). Other phenyl ring conformations result in steric hindrance in the binding site.

At this point there are three possible binding modes for the N^2-phenylguanines. Which is most probably the correct one? A computational experiment has been designed to answer this question.

Materials and methods

This section describes the steps followed to obtain the structures used in the computational experiments. The program Quanta-CHARMm [8] running on a Silicon Graphics Power Challenge workstation equipped with 3D viewing has been used.

Force field. The CHARMm 22.3 force field [9] was used throughout the present work. The atomic charges was generated by the Gasteiger–Hückel method. There is no explicit hydrogen bond term in the force field. The hydrogen bonds are computed implicitly as electrostatic nonbonded interactions in the force field. A dielectric constant equal to one was used.

Structure preparation. The first step was to clean up the crystallographic structure as a preparation for

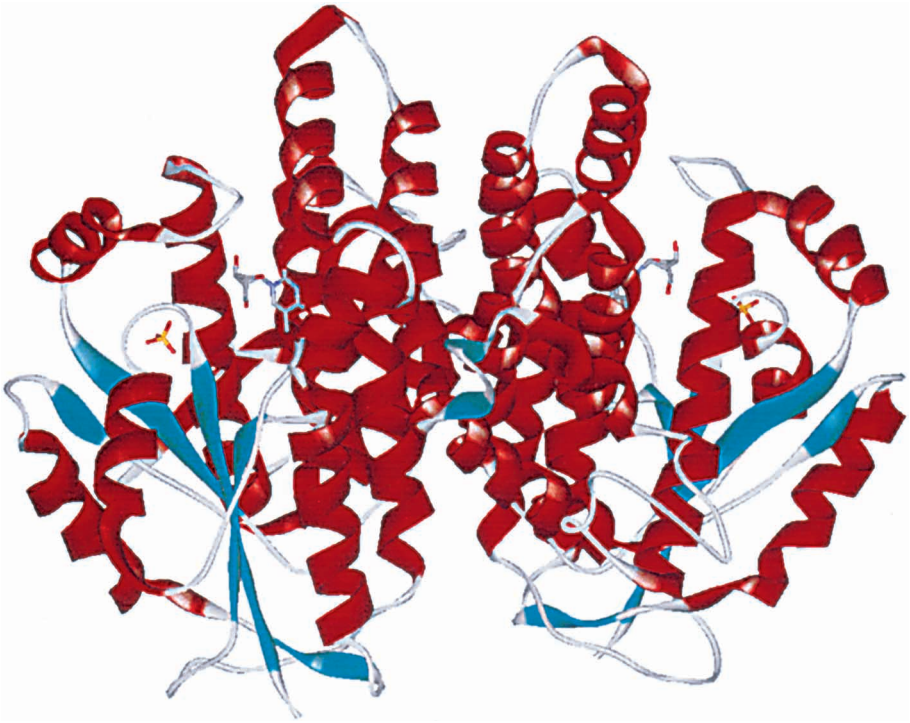

Figure 2. Model of the crystal structure of HSV1 TK in complex with deoxythymidine. The enzyme has a dimeric structure and, therefore, has two active sites per structure. The presence of a sulphate ion near the deoxythymidine binding site is a consequence of the use of ammonium sulphate during the crystallisation process.

molecular mechanics calculation. The sulphate ions and the water molecules were eliminated from the structure. In the original crystallographic structure of HSV1 TK, the first 45 and the last two amino acids were missing. The internal amino acid sequences 73–76, 150–151 and 263–277 were also missing. As the amino terminal amino acid sequence has no detectable influence on the catalytic activity [7] and the missing carboxy terminal sequence has only two amino acids, these sequences have been left out. The internal missing amino acid sequences are part of surface loops located far away from the active site. They were rebuilt using the Protein Design module of the Quanta program. The algorithm of loop reconstruction is based on searching loop fragments in an internal databank that contains loops from many kinds of enzymes. The program tries to fit all stored loops that have the correct number of missing amino acids in the space where the original loop should be, using its free ends as anchors. A number of possible loops is selected and the operator needs to choose one. The choice should be based on statistical parameters and on the visual aspects, to avoid possible overlaps between the loop and the existing structure of the enzyme. In this way, the loop Gly

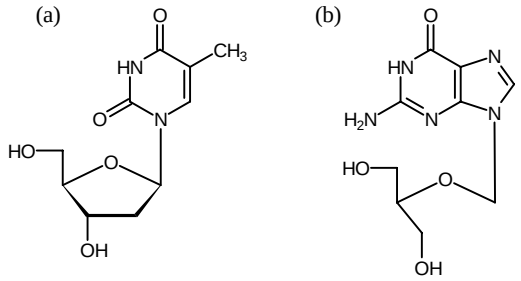

Figure 3. (a) 2′-Deoxythymidine; (b) Ganciclovir.

73–Asp 76 has been selected from the enzyme 2OVO, starting on Cys 24, the loop Ser 150–His 151 from 3CYT, starting on Thr 64 and the long loop Ser 263–Asn 277 from 5CHA, starting on Thr 200. After being connected to the free ends, the chosen loops and some residuals from the anchors are submitted to 500 steps of steepest descent minimisation in order to reduce the tension in the enzyme structure.

The missing hydrogen atoms have also been added and the charged residues were adjusted to the iso-

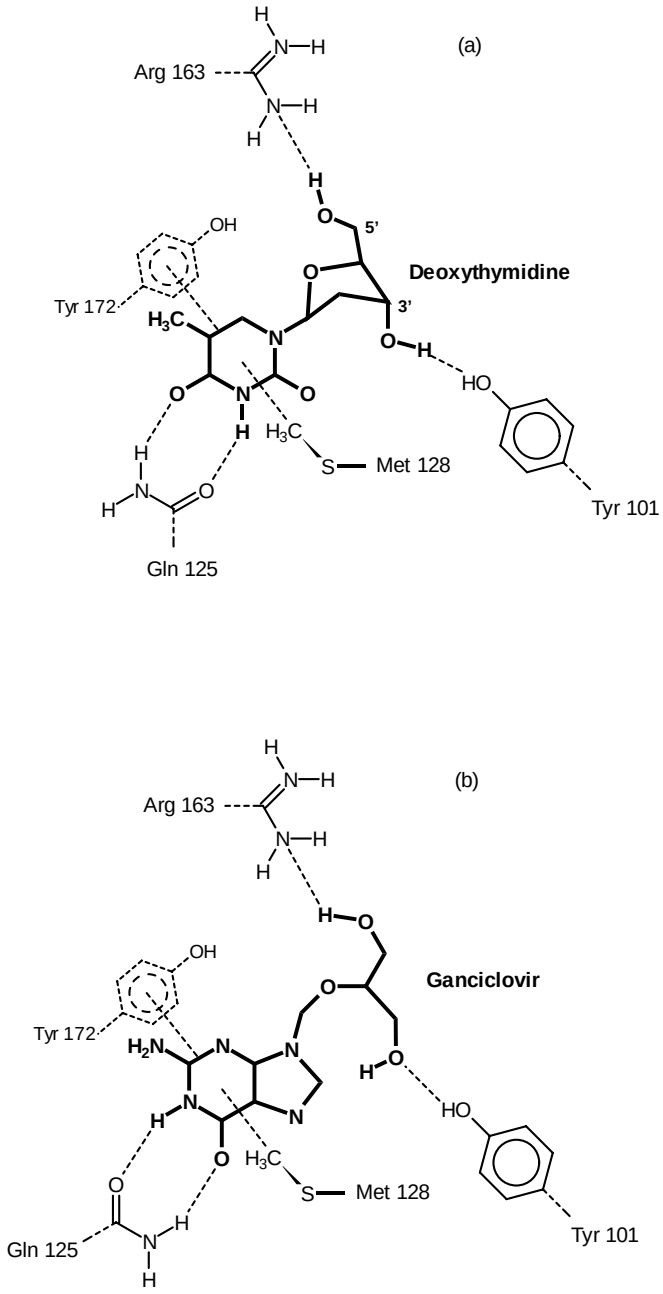

Figure 4. Schematic representation of (a) deoxythymidine and (b) ganciclovir molecules in the HSV1 TK active site. The amino acid residue Tyr 172 is drawn as a dashed line to indicate its position below the plane of the paper. Met 128 is above the plane of the paper and its SCH_3 group is pointing to the purine/pyrimidine ring of the ligand molecule. The dashed lines between the ligand molecule and the amino acid residues represent non-bonding interactions. Some atoms and bonds of deoxythymidine and ganciclovir have been omitted for clarity.

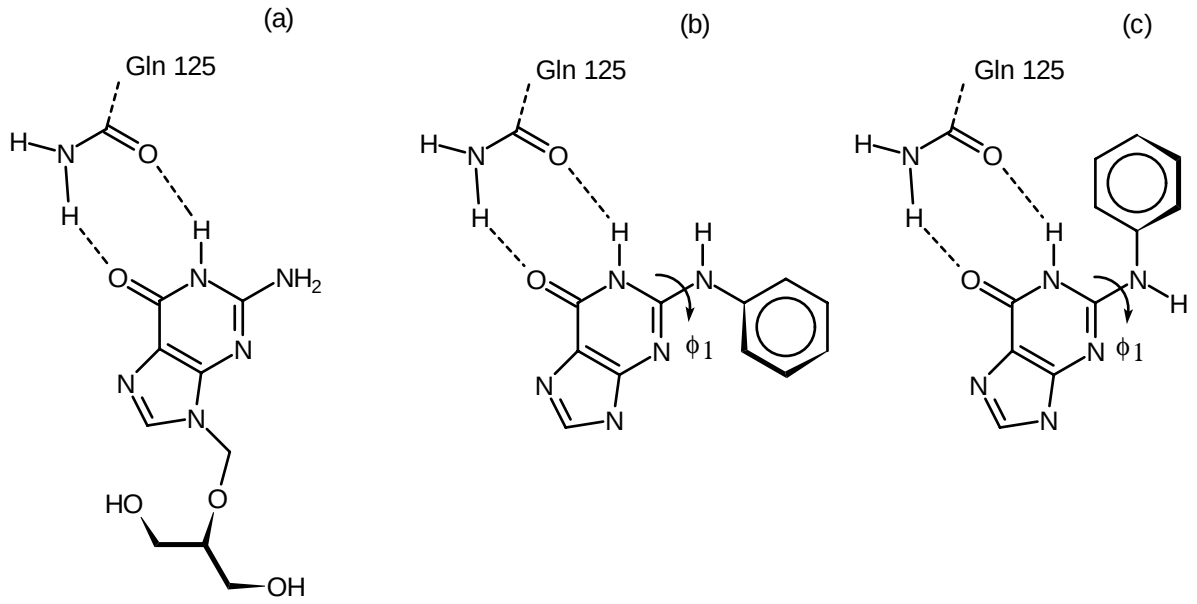

Figure 5. The ganciclovir-like binding mode of N^2-phenylguanines. (a) The ganciclovir binding mode has been used as template; (b) binding mode of N^2-phenylguanines in which the torsion angle ϕ_1 is 0° and (c) 180°.

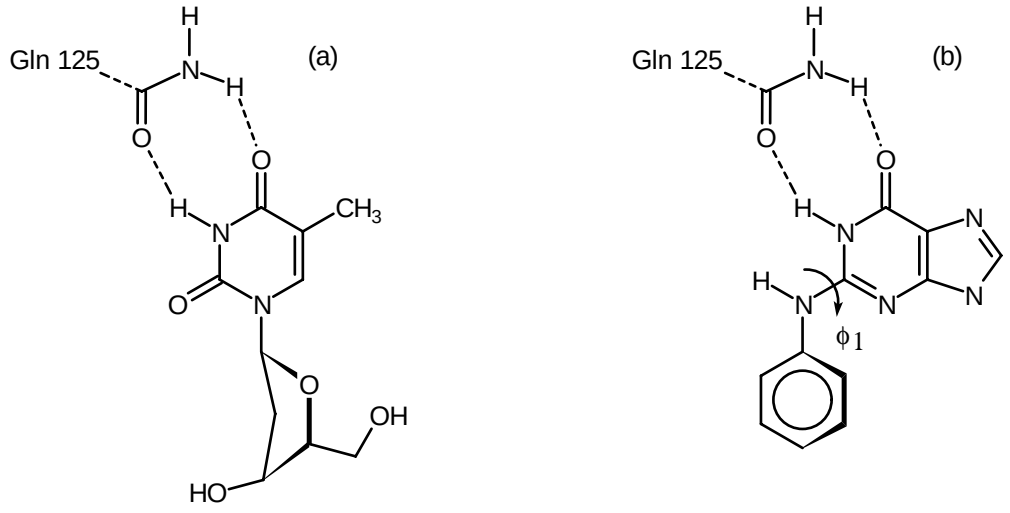

Figure 6. The deoxythymidine-like binding mode of N^2-phenylguanines. (a) The deoxythymidine binding mode has been used as template; (b) binding mode of N^2-phenylguanines in which the torsion angle ϕ_1 is 180°. The conformation in which this angle is 0° is not permitted.

electric point. The crystallographic structures of deoxythymidine and ganciclovir molecules were cleaned up using the Molecular Editor module of Quanta. The structures of p-H, p-F, p-Cl, p-Br and p-I-N^2-phenylguanine were built using the ChemNote module of Quanta and manually adjusted to match the spatial position of deoxythymidine and ganciclovir in the HSV1 TK active site. CHARMm 22.3 force field parameters were assigned to the inhibitor molecules.

System definition. The system submitted to the molecular dynamics simulation included only the inhibitor molecule and the complete amino acid residues in which at least one of their atoms was less than or equal to 8 Å distant from the nearest inhibitor atom. The carbon and nitrogen atoms (atom types C, N and CA) from the enzyme backbone have been submitted to a harmonic constraint potential in which the force constant was equal to 100 kcal mol^{-1} $Å^{-2}$. As the HSV1 TK crystallographic structure is a dimer, two active sites were included in the simulation.

Molecular dynamics. Fifteen molecular dynamics simulations were performed, which correspond to the five N^2-[substituted]-phenylguanines in each of the three possible binding modes. Each simulation was performed as follows: (a) 1000 steps of steepest descent minimisation in which the inhibitor molecule was kept frozen; (b) 1000 steps of conjugate gradient minimisation on the whole system; (c) warm up from 0 to 310 K over 4 ps, with temperature increase every 0.05 ps; (d) equilibration at 310 K over 4 ps with atomic velocity assignment every 0.05 ps; (e) free simulation at 310 K over 8 ps; (f) quenching to 0 K over 4 ps with atomic velocities assignment every 0.05 ps; (g) 1000 steps of steepest descent minimisation on the whole system; and (h) 1000 steps of conjugate gradient minimisation on the whole system.

$\Delta\Delta H_{EL}$ *calculation.* The same system defined for the molecular dynamics calculation was used in the calculation of the $\Delta\Delta H_{EL}$ values. In a first step, the ΔH_{EL} values for p-H, p-F, p-Cl, p-Br and p-I-N^2-phenylguanine, in the three binding modes considered, were calculated according to the expression $\Delta H_{EL} = H_{EL} - (H_E + H_L)$. In this expression, H_{EL} is the total CHARMm energy of the enzyme-ligand system, H_E is the total CHARMm energy of the active site in the same conformation, but with no ligand, and H_L is the total CHARMm energy of the ligand molecule only, in the same conformation as when it was bound to the active site. For each one of the three binding modes used in the computational experiment, five values of ΔH_{EL} were calculated. In each binding mode, a zero value was attributed to the smallest ΔH_{EL} and the difference $\Delta\Delta H_{ELi} = \Delta H_{ELi} - \Delta H_{ELsmallest}$ for the *i*th compound was computed.

Human TK and HSV1 TK alignment. The amino acid sequences of the HSV1 TK (375 amino acids) and human TK (234 amino acids) were aligned through the algorithm of Multal Data Matrix [10], implemented in the program Cameleon [11]. The total identity percentual was computed manually by counting the exact amino acid matches along the two sequences and comparing the number of matches with the total number of amino acids in the human TK (51 matches in 234 amino acids, 21%). The functional identity percentual was computed in a similar way, but now by counting the amino acid matches according to the following criteria of amino acid classification: acidic (Asp, Glu), hydrophobic (Ala, Gly, Leu, Ile, Val), amide (Asn, Gln), aromatic (Tyr, Phe, Trp), basic (Arg, Lys, His), hydroxy (Thr, Ser), sulfur (Met, Cys) and proline (Pro).

Computational experiment

The computational experiment was designed to give an indication on the correct binding mode of N^2-phenylguanines. The experiment has two objectives. The first one is to check some qualitative binding parameters in order to verify the visual aspect of the HSV1 TK–N^2-phenylguanine interaction and to make a comparison with the deoxythymidine and ganciclovir binding modes. The second objective is to calculate the HSV1 TK–N^2-phenylguanine interaction energies in order to correlate them with the respective inhibitory activity values (log $1/IC_{50}$). A linear relationship between log ($1/IC_{50}$) and the calculated interaction energy should be expected [12] for a set of N^2-phenylguanine derivatives in the correct binding mode. The experiment encompassed a small set of N^2-[substituted]-phenylguanines, binding to the active site in the three possible modes discussed in the previous section.

The qualitative binding parameters to be checked are: (a) the stability of the double hydrogen bond between the inhibitor molecule and Gln 125; (b) the degree of parallelism between the benzene ring of Tyr

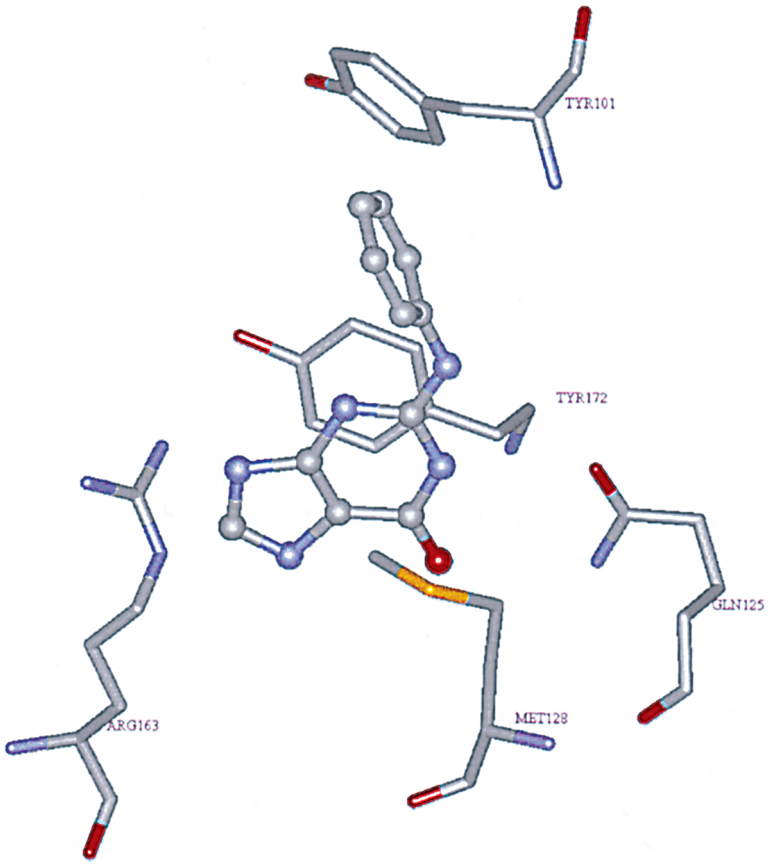

Figure 7. Proposed binding mode of N^2-phenylguanines. The hydrogen atoms were omitted for clarity. The N^2-phenylguanine molecule is represented as a ball and stick model.

172 and the guanine ring of the inhibitor; (c) the direction to which the SCH_3 group of Met 128 is pointing; and (d) the environment around meta and para positions of the inhibitor phenyl ring. The first three parameters are expected to be in accordance with Figure 4. The environment around the meta and para positions of the phenyl ring requires further consideration.

Equation (1) is a recalculated version (Gaudio and Takahata, unpublished results) of Equation (1d) presented by Wright et al. [5] as the result of a QSAR study on a set of 36 N^2-[substituted]-phenylguanines as competitive inhibitors of HSV1 TK. Equation (1) was derived after updating Wright's parameter set [5] following Hansch et al. [13].

$$\log(1/IC_{50}) = 0.59(\pm 0.23)\pi_m - 0.41(\pm 0.20)\pi_p + 1.05(\pm 0.75)\sigma_m + 2.38(\pm 0.87)R_p + 5.07(\pm 0.24), \quad (1)$$

$$n = 34,\ r = 0.897,\ s = 0.399,\ F = 29.77.$$

In this equation, IC_{50} is the drug molar concentration that produces 50% of inhibition of the enzyme activity; π_m and π_p are the Hansch–Fujita hydrophobic parameters at positions meta and para of the phenyl ring; σ_m is the electronic Hammett substituent constant at the meta position; R_p is the Swain–Lupton resonance parameter at the para position [12]. The numbers in parentheses are the coefficient 95% confidence limits; n is the number of compounds considered in the equation; r is the correlation coefficient; s is the standard deviation and F is the variance ratio. According to Equation (1), the inhibitory activity of N^2-[substituted]-phenylguanines on HSV1 TK is increased by the presence of a hydrophobic substituent at the meta position and a hydrophilic substituent at the para position. At the same time, the inhibitory activity is increased by the presence of a substituent that withdraws electrons through an inductive effect on the meta position and through a resonance effect at the para position of the phenyl ring. As, in general,

substituents that withdraw electrons through inductive effect are polar and hydrophobic substituents are apolar we would expect an amphiphilic environment in the neighbourhood of the meta position in the binding site. A hydrophilic environment around the para position is also expected.

The aim of the drug–receptor interaction energy calculation experiment is to compare the interaction energy of a given series of compounds, calculated for each one of the binding modes discussed in the previous section, with their respective log ($1/IC_{50}$) values. The theoretical basis of this experiment is as follows. Let us consider the general enzyme-ligand binding process:

$$L\text{- - -}xH_2O + E\text{- - -}yH_2O \longrightarrow E\text{- - -}L\text{- - -}zH_2O + (x + y - z)H_2O$$

where L is the ligand, E is the enzyme, x, y and z are numerical coefficients and the dashed line denotes non-bonding interactions. According to thermodynamics, the enzyme-ligand binding free energy change (ΔG_{bind}) is given by Equation 2,

$$\Delta G_{bind} = \Delta H_{bind} - T\Delta S_{bind}, \quad (2)$$

in which ΔH_{bind} is the binding enthalpy change, T is the absolute temperature and ΔS_{bind} is the binding entropy change. If a given series of compounds had ΔS_{bind} approximately constant, then Equation (3) will follow.

$$\Delta\Delta G_{bind} \cong \Delta\Delta H_{bind} \quad \text{(in the series)}. \quad (3)$$

The binding enthalpy change can be written as in Equation (4),

$$\Delta H_{bind} = \Delta H_{EL} + \Delta H_{solv} + \Delta H_{conf}, \quad (4)$$

in which ΔH_{solv} and ΔH_{conf} are the enthalpy changes due to the non-bonding interactions between the solvent and the ligand/enzyme and due to the conformational changes, respectively. ΔH_{EL} is the enthalpy change due to the non-bonding interactions between the enzyme and the ligand. Furthermore, if the same series of compounds had ΔH_{conf} and ΔH_{solv} approximately constant, then Equation (5) will also follow.

$$\Delta\Delta H_{bind} \cong \Delta\Delta H_{EL} \quad \text{(in the series)}. \quad (5)$$

Considering all the above approximations at the same time, then Equation (6) can be applied.

$$\Delta\Delta G_{bind} \cong \Delta\Delta H_{EL} \quad \text{(in the series)}. \quad (6)$$

It is very difficult to find a series of N^2-phenylguanine derivatives on which all the above approximations can be applied. Considering the set of 36 N^2-[substituted]-phenylguanine derivatives studied by Wright et al. [5], the closest series of compounds one can accept as being considered in such approximations is the subset of para monoatomic substituted derivatives; H, F, Cl, Br and I. In this series one could expect similar conformational changes for the five compounds, which would make $\Delta\Delta H_{conf} \cong 0$ (in the series). However, the approximation $\Delta\Delta S_{bind} \cong \Delta\Delta H_{solv} \cong 0$ is crude, because of the increasing substituent volume along the series. We are considering that the solvent accessible surface area of the substituent is not very large when compared to the surface area of the whole ligand molecule. In fact, the solvent accessible surface area of *p*-iodine-N^2-phenylguanine (484.5 $Å^2$, calculated through the method of Gaudio and Takahata [14]) is approximately 10% higher than the surface area of the unsubstituted N^2-phenylguanine (439.9 $Å^2$). This would keep the values of $\Delta\Delta S_{bind}$ and $\Delta\Delta H_{solv}$ at an acceptable level for the purpose of our approximations. Unfortunately the present method cannot be applied to deoxythymidine and ganciclovir molecules. The structural differences between these molecules and the para monoatomic substituted N^2-phenylguanines are beyond the approximations discussed above.

Results

The results of the qualitative part of the computational experiment are shown in Table 1. The results are presented in terms of an average over the p-H, p-F, p-Cl, p-Br and p-I-N^2-phenylguanine. As an example, in the analysis of the formation of the double hydrogen bond between Gln 125 and the inhibitor molecule (binding mode according to Figure 5a) in the active site A of HSV1 TK, one can see that the result is **1**/2. This means that only half of the double hydrogen bond has been formed at the site A in the majority of the five structures analysed. The results presented in Table 1 point to a deoxythymidine-like binding mode as the binding mode adopted by N^2-phenylguanine inhibitors. Considering the similarity between N^2-phenylguanines and ganciclovir molecules, one might expect that N^2-phenylguanine derivatives should adopt the ganciclovir-like binding mode. The indication of deoxythymidine-like binding mode for N^2-phenylguanine derivatives initially

Table 1. Results of the qualitative part of the computational experiment. The average qualitative binding characteristics of p-H, p-F, p-Cl, p-Br and p-I-N^2-[substituted]-phenylguanine, in the three possible binding modes, can be compared to the expected results

Binding mode	Site[a]	Gln 125[b]	Met 128[c]	Tyr 172[d]	meta[e]	para[e]
Expected	A	**2/2**[f]	**Yes**	**Yes**	**Amphi**	**Hydro**
	B	**2/2**	**Yes**	**Yes**	**Amphi**	**Hydro**
Ganciclovir like[g] ($\phi_1 = 0°$)	A	**1**/2	**Yes**	No	**Amphi** Lipo	Lipo
	B	**1**/2	No	**Yes**	Lipo **Amphi**	Lipo
Ganciclovir like[h] ($\phi_1 = 180°$)	A	0/2	**Yes**	No	Lipo Lipo	Lipo
	B	0/2	No	**Yes**	Lipo Lipo	Lipo
d-thymidine like[i] ($\phi_1 = 180°$)	A	**1**/2	No	**Yes**	**Amphi** **Amphi**	**Hydro**
	B	**1**/2	**Yes**	**Yes**	**Amphi** Hydro	**Hydro**

[a] HSV1 TK binding site under analysis.
[b] Double hydrogen bond between Gln 125 and the inhibitor molecule. 2/2 = double hydrogen bond formed; 1/2 = only one hydrogen bond formed; 0/2 = no hydrogen bond formed.
[c] Direction to which the SCH_3 group of Met 128 is pointing. Yes = pointing to the guanine ring of the inhibitor molecule; No = pointing to other direction.
[d] Spatial position of the benzene ring of Tyr 172. Yes = parallel to the guanine ring of the inhibitor molecule; No = not parallel to the guanine ring.
[e] Environment of the active site in the neighbourhood of meta and para position. In the meta column, there are two designations for the environment because of the two meta positions of the phenyl ring. Hydro = hydrophilic; Lipo = lipophilic; Amphi = amphiphilic.
[f] Bold typeface has been used to highlight the comparison between the expected and the obtained results.
[g] See Figure 5b.
[h] See Figure 5c.
[i] See Figure 6b.

Table 2. Values of the change in the enzyme-ligand interaction enthalpy change ($\Delta\Delta H_{EL}$) along the series p-H, p-F, p-Cl, p-Br and p-I-N^2-phenylguanine

Inhibitor	log $1/IC_{50}$[b]	$\Delta\Delta H_{EL}$/kcal mol^{-1} [a]		
		Ganciclovir-like ($\phi_1 = 0°$)	Ganciclovir-like ($\phi_1 = 180°$)	Deoxythymidine-like ($\phi_1 = 180°$)
p-Br	**6.00**[c]	26.0	0.0	**0.0**
p-H	**5.10**	4.7	28.2	**9.7**
p-I	**4.40**	0.0	21.3	**21.3**
p-Cl	**4.00**	27.1	25.3	**24.7**
p-F	**3.52**	12.4	17.9	18.0

[a] In computing the $\Delta\Delta H_{EL}$ values, a zero was assigned to the smaller value of ΔH_{EL} along the series.
[b] Taken from reference [5].
[c] Bold typeface has been used to highlight the correlation between log $1/IC_{50}$ and $\Delta\Delta H_{EL}$ values.

seemed unexpected. The proposed binding mode of N^2-phenylguanine derivatives is sketched in Figure 7.

Table 2 shows the change in the enzyme-ligand interaction enthalpy change ($\Delta\Delta H_{EL}$) along the series p-H, p-F, p-Cl, p-Br and p-I-N^2-phenylguanine. Once again the deoxythymidine-like binding mode appears to be the adopted mode for the N^2-phenylguanine derivatives. The correlation coefficients between the $\Delta\Delta H_{EL}$ values for each one of the three binding modes and log $1/IC_{50}$ are 0.19 for ganciclovir-like (phenyl ring 0°), 0.58 for ganciclovir-like (phenyl ring 180°), and 0.88 for deoxythymidine-like (phenyl ring 180°). This last correlation coefficient becomes 0.99 if the p-F derivative is not considered. The fact that high inhibitory activity values are related to small $\Delta\Delta H_{EL}$ values is consistent with the fact that a stronger enzyme-inhibitor interaction, which produces higher inhibitory activity, is associated to smaller ΔH_{EL} values. The deviation in the $\Delta\Delta H_{EL}$ values of the p-F derivative from the linear relationship can be related to the anomalous behaviour of the entropy of solvation presented by many fluorinated compounds [15].

Experimental indications of the proposed binding mode

The N^2-phenylguanosine derivatives studied by Wright et al. [3], i.e., N^2-phenyl-2′-deoxyguanosine (Figure 8), presented an interesting feature; although very similar to ganciclovir, they proved not to be phosphorylated during interaction with the HSV TK active site. If the N^2-phenylguanosine derivatives bound to the HSV1 TK active site adopting the ganciclovir-like binding mode, then there would be no reason why they should not be phosphorylated during the interaction. Superimposing the guanine ring of N^2-phenylguanosine on the guanine ring of the ganciclovir molecule in the crystallographic structure, the O5′ atom of N^2-phenylguanosine can be positioned only 0.5 Å from the corresponding oxygen atom of the ganciclovir molecule, which would not affect the phosphorylation process. However, when the guanine ring of N^2-phenylguanosine is positioned according to the deoxythymidine-like binding mode, the nearest spatial position at which its O5′ atom can be positioned from the O5′ atom of deoxythymidine is about 2.8 Å. In computing this distance, the energetic aspects of the conformation of the sugar ring of N^2-phenylguanosine have not been considered, which means that the distance between the two O5′ atoms could be even higher. Such a large distance from the ideal phosphorylation position could prevent the interaction between O5′ and gamma phosphate of ATP with subsequent inhibition of phosphorylation.

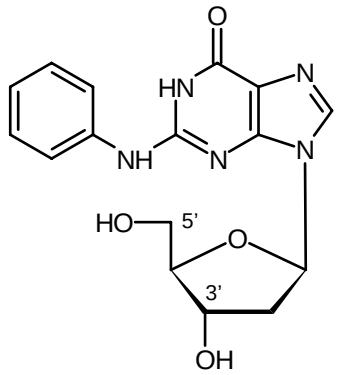

Figure 8. N^2-Phenyl-2′-deoxyguanosine.

Conclusions

Both the qualitative and the quantitative parts of the computational experiment point in the same direction. The N^2-phenylguanine derivatives bind to the HSV1 TK active site adopting the deoxythymidine-like binding mode. The experimental observation about the nonphosphorylative interaction between N^2-phenylguanosine derivatives and HSV1 TK gives additional support to this hypothesis.

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