**Original Research Article**

*In-silico* designing of NKK: A better ligand than Aciclovir against Herpes Simplex Virus

Neelabh\(^1\), K. K. Jeswara\(^2\), A. Kumari\(^1\), K. Singh\(^*\)

\(^1\)Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005, India

\(^2\)Department of Bioinformatics, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005, India

**ARTICLE INFO:**

*Article history:*
Received: 30 October 2014
Received in revised form: 18 November 2014
Accepted: 28 November 2014
Available online: 31 March 2015

*Keywords:* Aciclovir, Herpes Simplex Virus, in-silico, thymidine kinase

**ABSTRACT**

Aciclovir is the best drug known till date against the herpes simplex virus (HSV). It targets an enzyme, thymidine kinase made by the HSV. In the current study we have successfully designed a drug candidate “NKK”, which is far more efficient than aciclovir in terms of binding energy and physicochemical properties against the same target i.e. thymidine kinase. This *in-silico* study utilizes three commonly used softwares viz. Autodock 4.0, FireDock and Hex dock in order to demonstrate that “NKK” is a better ligand than aciclovir with respect to aforesaid properties.

**General Structure of Herpes Simplex Virus**

HSV in general comprises of an outer membrane lipid envelop that has a large number of glycoproteins. This lipid envelop houses the capsid which is icosahedral in shape. It has a diameter of 100 nm and a large double stranded DNA genome having a toroidal shape present inside the capsid. This icosahedral capsid comprises of capsomeres and in turn is surrounded by an amorphous proteinaceous coat called tegument containing additional viral proteins [3]. An image of HSV virus depicting its structural components have been provided in Fig. 1 (Picture redrawn from http://www.bio.davidson.edu/people/sosaurafova/assets/bio307/jehodg/e/page01.html) [4].

![Structure of the Herpes Simplex Virus](image_url)

**Fig. 1: Structure of the Herpes Simplex Virus**

*Corresponding Author: Dr. Karuna Singh, Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005 India. E-Mail: karunasinh5.bhu@gmail.com*
Although the diseases caused by HSV are generally non-fatal but Keratitis (corneal infection) and the infection of Central Nervous System (CNS) or encephalitis can be highly pathological. Other symptoms include tingling, itching or burning, formation of fluid filled sores, fever, muscle aches, swollen lymph nodes and trouble in urinating [5].

Aciclovir is currently the best drug available for the treatment of herpes simplex virus targeting the enzyme thymidine kinase of the virus [6]. The mechanism of action of aciclovir has been shown in Fig. 2

![Mechanism of action of aciclovir](image)

**Fig. 2: Mechanism of action of aciclovir**

Through this depiction we wish to show that aciclovir targets the enzyme thymidine kinase of the virus thus forming a complex. Aciclovir present in this complex through a series of reactions gets converted to aciclovir tri-phosphate which is responsible for inhibiting the viral DNA polymerase hence the replication of the virus. Furthermore, this aciclovir triphosphate has also been reported to inhibit the nucleotide deoxyguanosine triphosphate.

Although aciclovir is the best known drug till date but it has many side effects such as nausea, dizziness, drowsiness, kidney problems, mood changes, shaky/unsteady movement and difficulty in speaking. Some life threatening disorders have also been reported due to this medication especially in the cases where the immune system is compromised. Some other symptoms that are visible as the side-effects of the aciclovir treatment are fatigue, arrhythmia, easy bruising/bleeding, blood (dark) coloured urine, severe stomach/abdominal pain, yellowish eyes, sudden vision changes, loss of consciousness etc. [7]

Moreover, the data of a 10 year long study suggests that there has been a marked increase in resistance against the most potent drug aciclovir amongst the patients of HSV. This study suggests that in the case of immuno-competent patients an increased resistance of 0.5% has been observed while in the
case of immuno-compromised patients the condition is worse indicating 2.5-10% increase [8].

The present paper proposes a new ligand (NKK) that has a better binding efficiency to the viral thymidine kinase as compared to aciclovir. The results of its binding efficiency have been checked through blind docking and specific docking approaches. Three softwares have been used for the purpose of docking. They are Autodock 4.0, FireDock and HexServer. Running on different algorithms and utilizing different parameters for prediction of the binding efficiency all the 3 softwares unanimously predicted that the ligand “NKK” has a better binding efficiency than aciclovir.

Materials and Methods

Softwares and Databases Used

A number of softwares and databases have been used in this study for designing the ligand and docking.

Protein Data Bank

The Protein Data Bank as the name suggests comprises of the 3D structures of large biological molecules. Apart from the data obtained for the proteins it also provides information about the nucleic acids. The data obtained from the crystallographic studies is stored in a uniform format of atomic co-ordinates and partial bond connectivities in this data bank [9]. PDB holds its importance primarily because of the large collection it has and secondly because of the free availability and regular upgradation of the database. It can be accessed from the following link http://www.rcsb.org/ [10].

Discovery Studio Visualizer

Discovery studio Visualizer is a powerful software for the analysis of various biological molecules and inferring useful information from them [11].

ACD/Chemsketch

ACD/Chemsketch is a freely available software package that allows one to draw the chemical sketch of various compounds such as organics, organometallics, polymers and Markush structures. Apart from drawing the structures one can easily determine the properties such as the molecular weight, density, molar refractivity etc. It can be accessed from the following link (http://www.acdlabs.com/resources/freeware/chemsketch/) [12].

Open Babel

Open Babel [13] is free software that translates the various chemical formats into each other. It can be accessed from the following link (http://openbabel.org/wiki/Main_Page) [14].

Autodock 4.0

Autodock is the premier free software package that is utilized worldwide for the docking purposes. It offers a variety of approaches such as Monte Carlo simulated annealing (SA), Genetic algorithm (GA) and a hybrid local search GA also called as Lamarckian Genetic Algorithm (LGA).

It is the most reliable amongst the docking softwares and has been utilized in this paper for docking. Site specific docking approach has been used through this software. By site specific docking it is meant that the docking will take place at a predestined place and not at any random place. It can be downloaded from http://autodock.scripps.edu/ [15].

FireDock

FireDock forms the first web server and proves its utility in the flexible refinement and scoring of protein-protein docking solutions. FireDock works in a way so as to optimize the side chain conformations and rigid body orientations and hence provides a high grade refinement. The user friendly interface and 3-D visualisation of results increases the utility of FireDock to a greater extent [16].

In the present study we have used FireDock in a blind docking approach. This means that the ligand will not bind in a predestined place but will bind to the best possible place as determined by the software.

HexServer

HexServer functions on the Fast Fourier based Transform (FFT) protein docking. Its unique feature is that it has been provided with two graphic processors that work simultaneously in order to speed up the process of docking (2 magnitudes faster) than the other FFT based approaches [17].

Blind docking approach is utilized in the same way in this case as in case of FireDock.

Molinspiration

Molinspiration, a self governing group, chiefly focuses on providing the scientific community with broad range of cheminformatics softwares tools that can be used for the molecule manipulation and processing. The tools include SMILES and SDfile conversion, molecule fragmentation, normalization of molecules, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, generation of tautomers, molecular database tools supporting substructure search or similarity and pharmacophore similarity search etc. It can be accessed online from http://www.molinspiration.com/ [18].

The Lipinski rule of five

Medicinal chemist Christopher Lipinski concluded from his work that a compound is more likely to be permeable and can be effortlessly absorbed by the body if the following criteria are met [19].

• The molecular weight of the compound is less than 500.
• Lipophilicity which is expressed as a quantity known as log P (the logarithm of the partition coefficient between water and 1-octanol), is less than 5.
• Hydrogen bond donors (usually the sum of hydroxyl and amine groups in a drug molecule) should be less than 5.
• Hydrogen bond acceptors (estimated by the sum of oxygen and nitrogen atoms) should be less than 10.
Methodology

The structure of thymidine kinase of the Herpes Simplex Virus conjugated with aciclovir (the drug currently prescribed against the virus) was retrieved from the PDB database (Structure ID: 2KI5; Herpes simplex type-1 thymidine kinase in complex with the drug aciclovir at 1.9 Å resolution) as shown in Fig. 3. The structure obtained from the PDB was subjected to processing through the Discovery Studio Visualizer software.

![Figure 3: Aciclovir conjugated with thymidine kinase](image)

The above mentioned software was utilized in order to delete the water molecules present in the structure. This software was used mainly to determine the active site of the protein to which the ligand in the complex was bound as shown in Fig. 4.

![Figure 4: Figure depicting the active site regions of protein thymidine kinase](image)

Protein thymidine kinase is a dimer therefore the ligand is bound to two positions in the protein. Next, it was also used to separate the protein and the ligand molecules as shown in Fig. 5.
Relevant literature and the related structures were studied in order to reach to a structure which was termed as “NKK”. ACD/ChemSketch was utilized to draw this structure and further optimization as shown in Fig. 6.

Throughout the study there was requirement for changing the format of the structures. This conversion between different formats was done through the Open Babel software. This new ligand has been compared to aciclovir with respect to its binding affinity for the protein thymidine kinase. This objective has been achieved through multiple softwares such

Fig. 5: Separate structure of protein thymidine kinase and ligand aciclovir

Fig. 6: Structure of new ligand designed termed as “NKK”
as HexServer and FireDock utilizing the blind docking approach and Autodock4.0 using the site specific docking approach. In the case of FireDock and HexServer input structures were of the protein (thymidine kinase) and the ligand (both aciclovir and NKK). Whereas, in the case of Autodock4.0 the site where aciclovir was previously bound was taken into account and grid was made across these sites only so as to ensure that the ligand binds to the same site. Both aciclovir and NKK were docked on the same site on thymidine kinase simultaneously. The Lamarckian genetic algorithm was utilised for arriving at the results.

Further the physiochemical properties of the ligand were analysed through the Molinspiration software which predicts if the ligand in question is following the Lipinski rule.

### Results and Discussion

Docking was performed through two different approaches. Firedock and HexServer used the blind docking approach whereas Autodock 4.0 used site specific docking approach. The results of the binding energy obtained by the three softwares for the interaction of the two ligands with the protein were compared. The results obtained through the Firedock have been provided in Table 1.

#### Table 1: Binding energies for the ligand aciclovir and NKK against the protein thymidine kinase as calculated by FireDock

<table>
<thead>
<tr>
<th>Structure</th>
<th>Global energy(binding energy)</th>
<th>Softened attractive van der Waals energy</th>
<th>Softened repulsive van der Waals energy</th>
<th>ACE(Atomic contact energy)</th>
<th>Insideness measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aciclovir best structure result</td>
<td>-40.16</td>
<td>-14.93</td>
<td>2.93</td>
<td>-12.68</td>
<td>0.26</td>
</tr>
<tr>
<td>NKK best structure result</td>
<td>-46.06</td>
<td>-18.61</td>
<td>1.12</td>
<td>-13.03</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Table 1 clearly displays that NKK has a lower negative value of Global energy (binding energy) than that of the aciclovir. Therefore it can be said that NKK binds better to the protein thymidine kinase than aciclovir. HexServer was also utilized for the purpose of docking. The protein along with the different ligands was given as the input every time and the other parameters were set as default. The program was set to find the complementarity of the shape. The results have been tabulated in Table 2. Through the table it is clearly evident that NKK has lower negative value for total energy than aciclovir. Hence, HexServer also shows that NKK has a better binding ability than aciclovir against thymidine kinase.

#### Table 2: Binding energies for the ligand aciclovir and NKK against the protein thymidine kinase as calculated by HexServer

<table>
<thead>
<tr>
<th>Structure</th>
<th>E total value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymidine Kinase and aciclovir</td>
<td>-198.42</td>
</tr>
<tr>
<td>Thymidine Kinase and NKK</td>
<td>-218.25</td>
</tr>
</tbody>
</table>

Autodock 4.0 was also utilized for docking. The results provided in Table 3 depict that NKK has a better free energy of binding than aciclovir thus strengthening our findings that NKK has a better binding affinity than aciclovir.

#### Table 3: Table depicting the results obtained through AutoDock 4.0

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Protein docked with aciclovir (best structure)</th>
<th>Protein docked with NKK (best structure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated Free Energy of Binding</td>
<td>-5.23 Kcal/mol</td>
<td>-7.48 Kcal/mol</td>
</tr>
<tr>
<td>Estimated Inhibition Constant, KI</td>
<td>146.49 µM</td>
<td>3.30 µM</td>
</tr>
<tr>
<td>Final Intermolecular Energy</td>
<td>-7.02 Kcal/mol</td>
<td>-8.67 Kcal/mol</td>
</tr>
<tr>
<td>vDW + Hbond + desolv Energy</td>
<td>-6.84 Kcal/mol</td>
<td>-8.18 Kcal/mol</td>
</tr>
<tr>
<td>Electrostatic Energy</td>
<td>-0.18 Kcal/mol</td>
<td>-0.49 Kcal/mol</td>
</tr>
<tr>
<td>Final Total Internal Energy</td>
<td>-0.71 Kcal/mol</td>
<td>-1.31 Kcal/mol</td>
</tr>
<tr>
<td>Torsional Free Energy</td>
<td>1.79 Kcal/mol</td>
<td>1.19 Kcal/mol</td>
</tr>
<tr>
<td>Unbound System's Energy</td>
<td>-0.71 Kcal/mol</td>
<td>1.31 Kcal/mol</td>
</tr>
</tbody>
</table>

The physiochemical properties of the compound NKK have been determined with the help of Molinspiration software (http://www.molinspiration.com/) and tabulated in Table 4. It is evident from Table 4 that “NKK” follows the Lipinski rule. For instance the MiLogP value of NKK is far below 5 which shows that it has good permeability across the cell membrane,
TPSA is below 160 and n violations = 0 shows that the compound can easily bind to the receptor. Molecular weight is less than 500, Nrotb is less than 5, Number of hydrogen bond donors is less than 5 (The sum of OHs and NHs), Number of hydrogen bond acceptors is less than 10 (The sum of Os and Ns).

Table 4: Physiochemical properties of NKK obtained through Molinspiration

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>miLogP</td>
<td>-2.231 (should be less than 5)</td>
</tr>
<tr>
<td>TPSA</td>
<td>133.24</td>
</tr>
<tr>
<td>Natoms</td>
<td>18.0</td>
</tr>
<tr>
<td>MW</td>
<td>254.202 (should be less than 500)</td>
</tr>
<tr>
<td>nON</td>
<td>9 (should be less than 10)</td>
</tr>
<tr>
<td>nOHNH</td>
<td>4 (should be less than 5)</td>
</tr>
<tr>
<td>Nviolations</td>
<td>0</td>
</tr>
<tr>
<td>Nrotb</td>
<td>1</td>
</tr>
<tr>
<td>Volume</td>
<td>198.648</td>
</tr>
</tbody>
</table>

Summary

The present study suggests and exemplifies the better binding capacity of the ligand NKK to thymidine kinase than aciclovir. An in-silico approach has been adopted for this purpose. Three softwares, Firedock, HexServer and Autodock 4.0 have been harnessed, Firedock and HexServer utilizing the blind docking approach and Autodock 4.0 utilizing the site specific docking approach. Furthermore, the physiochemical properties of NKK have been analysed through the Molinspiration software and it has been observed that it follows the Lipinski rule without any deviation which is of prime importance for production of a successful drug.

All the three mentioned softwares claim that NKK has a better binding ability towards thymidine kinase. Thus through this we can assume that NKK will follow the same mode of action that is followed by aciclovir and can be an effective drug against the Herpes Simplex virus in future.

Acknowledgement

One of the authors (Neelabh) gratefully acknowledges ICMR, New Delhi, India for providing the Junior Research fellowship.

Conflict of interest statement

We declare that we have no conflict of interest.

References


