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Comparative Molecular Docking Study on Thymidine Kinase (Herpes Virus) to Evaluate Drug Efficacy

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ABSTRACT

The present docking study was undertaken to comparatively evaluate the drug efficacy of few typical anti-herpes drugs (guanosine analogues) through their interaction with Herpes virus specific thymidine kinase enzyme. The nature of interaction between the drugs (ligands) and the enzyme (protein) was monitored by molecular modelling using two different docking protocols viz. CDOCKER and Lamarckian genetic algorithm. The docking results emphasizing on the hydrogen bonds and pi-interactions between the ligands and the protein along with their different binding energies were analysed. The binding energies were found to be within the range of -137.44 to -201.66 Kcal/mol using CDOCKER and -7.79 to -9.10 Kcal/mol using Lamarckian genetic algorithm respectively with the reference drugs. The least binding energy and hence the most efficacious drug was found to be that of Ganciclovir with a binding energy value of -201.66 Kcal/mol (CDOCKER) and -9.10 Kcal/mol (LAMARCKIAN GENETIC ALGORITHM) respectively. Thus the present study paves the way for development of newer congeners of Ganciclovir with improved activity and bioavailability.

Keywords: *Discovery Studio, Auto Dock, Exome Horizon, Docking, Thymidine kinase.*

INTRODUCTION

Herpes simplex virus (HSV) types 1 and 2 are ubiquitous organisms that cause infections in human populations throughout the world. The clinical manifestations of HSV infections are varied, ranging from asymptomatic disease to life-threatening illness in neonates and immunocompromised hosts [1]. They can infect and establish latency in the neurons of the sensory ganglia. Moreover, HSV can infect the central nervous system, causing meningitis and encephalitis. Viruses of the herpes group are morphologically indistinguishable, share many common features of intracellular development, but differ widely in biologic properties. All human herpes viruses (HHV) contain a large double stranded, linear DNA with 100-200 genes encased within an icosahedral protein capsid wrapped in a lipid bilayer envelope, called a virion. Following the binding of viral envelope glycoproteins to host cell membrane receptors, the virion is internalized and dismantled, allowing viral DNA to migrate to the host cell nucleus, where viral DNA replication and transcription occurs [2]. HSV-1 is predominantly associated with oral while HSV-2 with genital infection, both subtypes can infect cells at either site, even though reactivation of HSV-1 is more frequent in the oral whereas HSV-2 in the genital area [3]. The thymidine kinase (TK) of herpes simplex virus (HSV) is a multi-substrate enzyme [4] which possesses both TK and thymidylate kinase (TMPK) activities [5]. Herpes simplex virus type 1 (HSV 1) thymidine kinase (TK) is a multifunctional enzyme that possesses kinase activities normally performed by three separate cellular enzymes. It phosphorylates thymidine (dT), which is then transformed by cellular kinases to the triphosphorylated DNA building block, and deoxyuridine (dU); both reactions are comparable to the function of human cellular TK. Further, it converts deoxycytidine (dC) to deoxycytidine monophosphate (dCMP), as does human deoxycytidine kinase (dCK), and phosphorylates thymidylate (dTMP), as does human TMP kinase (TmpK) [6]. There is no permanent cure for these infections till date. Present day treatment involves the use of antiviral drugs to reduce the physical severity of outbreak-associated lesions and viral shedding, though this helps decreasing the chances of transmission to others only by maximum 50% [7]. There are two types of drugs that are clinically useful against HSV infections. The first category consists of nucleoside analogs like acyclovir and its prodrug valacyclovir, ganciclovir, penciclovir and its prodrug famciclovir, sorivudine and brivudine. These require phosphorylation by viral thymidine kinase to form triphosphates that are active inhibitors of viral DNA polymerase. The second category consists of direct viral DNA polymerase inhibitors like vidarbine, foscarnet and cidofovir [8]. Thus, both types of drugs target in dysfunctioning the replication centre i.e. DNA polymerase of the viral genome [9]. Viral latency is a problem in the management of HSV treatment. Lethal infections have also been reported in immunocompromised patients [10]. Treatment for primary oral or genital HSV infections in healthy patients is usually given orally for 7–10 days with acyclovir, valacyclovir, or famciclovir [11]. Treatment for recurrent disease can be episodic (treatment at the first sign or symptom of an outbreak) for 1–5 days to decrease the symptoms of HSV infection or suppressive (daily) to prevent recurrences [12, 13].

MATERIALS AND METHODS

Retrieval of 3D Structure:

The 3D structure of the protein was downloaded from RCSB (Research Collaboratory for Structural Bioinformatics), Protein Databank (PDB, http://www.pdb.org). The PDB ID of the selected protein was found to be 1KI3. The Water molecules and ligands attached to the protein were removed by using Swiss PDB Viewer. The Protein was having 620 no. of groups, 4691 no. of atoms and 4844 no. of bonds.

Structural Assessment of Protein:

The protein was sent for structural assessment to Exome Horizon. The Ramchandran Plot for all residue types is given in **Fig. 1**, Chi1-Chi2 plots, Main-chain parameters, Side-chain parameters, Residue properties, Main-chain bond length, Main-chain bond angles, RMS distances from planarity and distorted geometry were analyzed for input atom only [14].

Ligand Preparation:

The ligands were drawn using Moldraw tool of Exome Horizon in 2D and were converted into 3D before submission for docking. The ligands and their respective chemical structure and molecular formula are given in **Table 1.**

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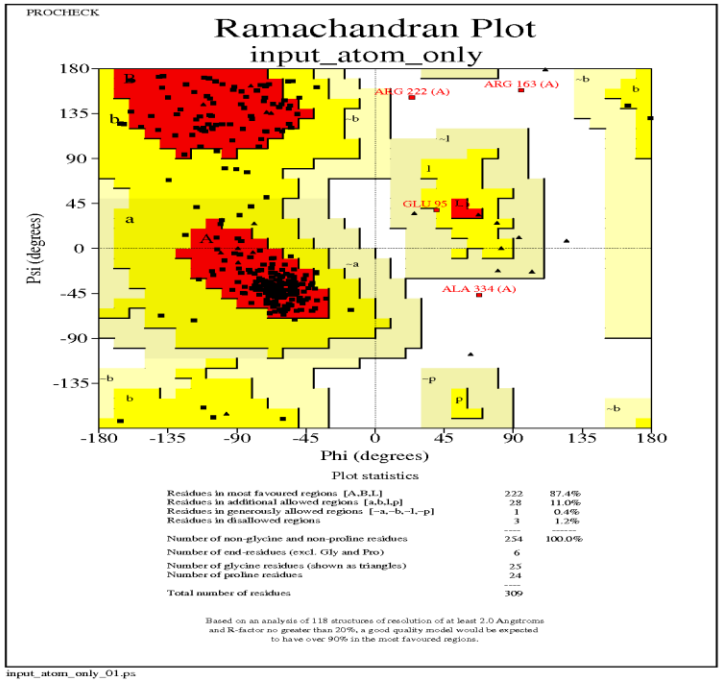
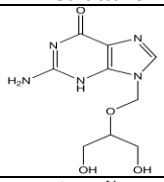
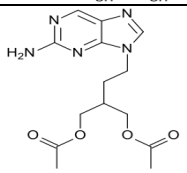
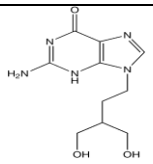
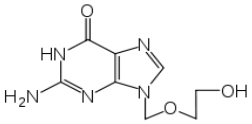
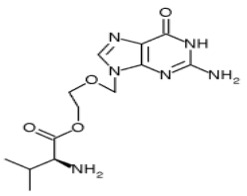


Fig. 1: Ramachandran plot analysis of Thymidine Kinase (Herpes Virus)

Table 1: Anti-herpes drugs under study

Sl. No.	Ligand Name	Mol. Formulae	2D structure
1	Ganciclovir	$C_9H_{13}N_5O_4$	
2	Famciclovir	$C_{14}H_{19}N_5O_4$	
3	Penciclovir	$C_{10}H_{15}N_5O_3$	
4	Acyclovir	$C_8H_{11}N_5O_3$	
5	Valacyclovir	$C_{13}H_{20}N_6O_4$	

Protein-Ligand Docking Studies:

Protein-ligand docking is used to check the structure, position and orientation of a protein when it interacts with small molecules like ligands. Protein-ligand docking aims to predict and rank the structures arising from the association between a given ligand and a target protein of known 3D structure. Protein-Ligand Docking module is further divided into different parts for user convenience like Receptor Preparation, Ligand Preparation, Binding Site Analysis, Dock and Analysis [15]. The protein-ligand docking was performed by CDOCKER, a molecular dynamics (MD) simulated-annealing-based algorithm [16] using Discovery Studio v3.1 and LAMARCKIAN GENETIC ALGORITHM with default parameter [17] using AutoDock 4.2 respectively to comparatively evaluate the binding efficacy of the drugs (under study) with the protein.

Binding Site Analysis:

Binding Site analysis is a fast detection program for the identification and visualization of possible binding sites and the

distribution of surrounding residues in the active sites. The centre of active site was chosen as grid map values for preparation of the grids. The spacing of grid was set to 1.00 ^{0}A and the no. of grid point was taken as 60 x 60 x 60 ^{0}A and protein-ligand docking were performed using Lamarckian genetic algorithm using default parameter [18]. The active sites are given in **Table 2**.

Table 2: Active sites and the centre of active sites of the protein 1KI3

Sl No.	Name of active sites	Residues in active sites	Centre of active sites
1	H1	HEYMYRYQRE	48.451, 83.610, 54.124
2	H2	PVTLTFAEM	45.561, 82.500, 35.901
3	H3	TQYPEPMDRME	56.100, 82.649, 48.235
4	H4	GSWRLDVAKR	24.472, 66.546, 54.825
5	H5	QITMGMPYAVPALFL	37.024, 84.058, 46.160
6	H6	VYVPEPPYATDIFDRHP	46.099, 77.310, 43.946
7	H7	WRWDLYNA	18.515, 73.498, 61.835
8	H8	QLAFVALNVFAWAL	24.263, 82.403, 52.439
9	H9	DGPMVLGALIRVYGLAFL	46.252, 70.229, 60.314

RESULTS

The ligands (drugs under study) were successfully docked into the binding pocket of the protein using docking protocols viz. CDOCKER, a molecular dynamics (MD) simulated-annealing-based algorithm and Lamarckian genetic algorithm respectively. The binding energies were observed in the range of -137.44 to -201.66 Kcal/mol using CDOCKER, and -7.79 to -9.10 Kcal/mol using LAMARCKIAN GENETIC ALGORITHM. The binding energies of the selected ligands obtained after docking with both the protocols are plotted in the graphs and from them **(Fig. 2 and Fig. 3)** the binding energies of all the active sites are observed, amongst which the best ligand which shows better activity in all the active sites using both the docking protocols was found to be Ganciclovir. The docked pose (interaction) of the best fit ligand (Ganciclovir) with definite amino acids (protein) obtained using the two protocols are given in **Fig. 4** and **Fig. 5** respectively.

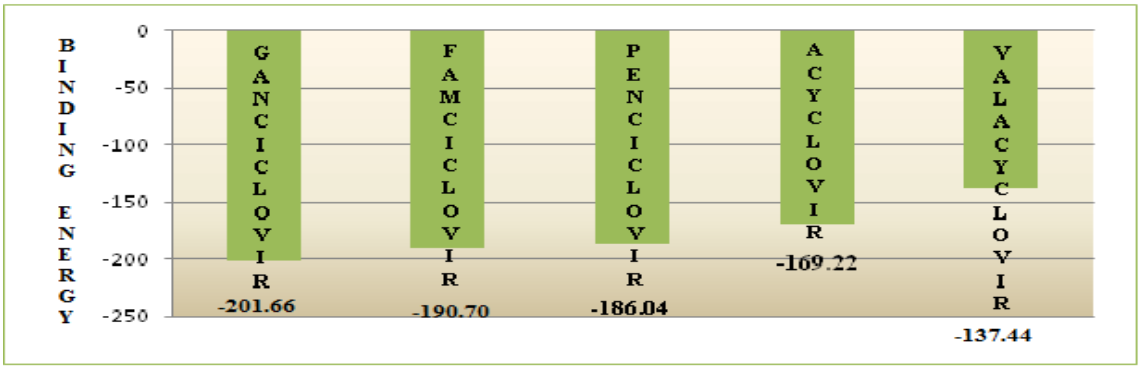


Fig 2: Binding energies of the drugs against thymidine kinase (herpes virus) using CDOCKER (Discovery Studio)

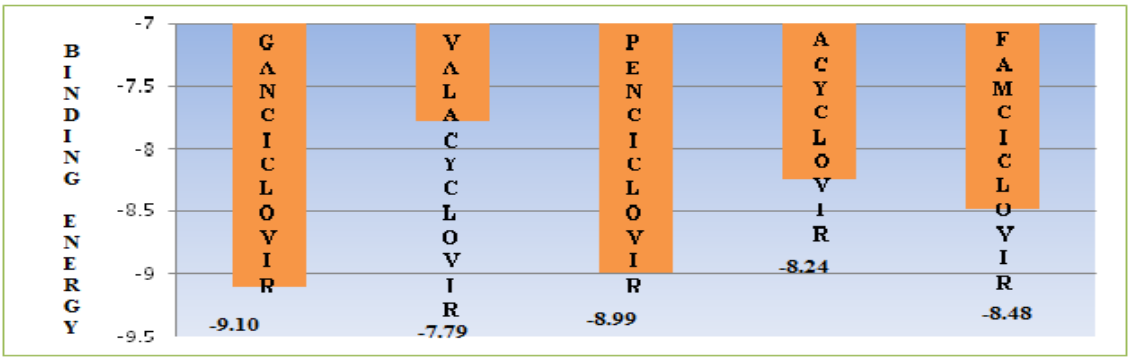


Fig 3: Binding energies of the drugs against thymidine kinase (herpes virus) using LAMARCKIAN GENETIC ALGORITHM (AutoDock)

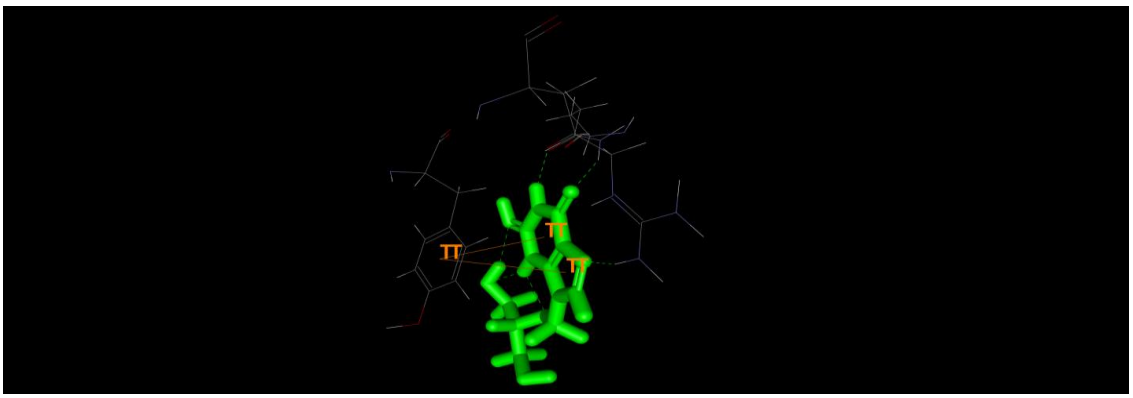


Fig. 4: Interaction of Ganciclovir against the protein 1KI3 using CDOCKER (DiscoveryStudio). The thin dotted lines with colours ● and ● Represent interacting hydrogen bonds and pi-interactions between the drug and the protein respectively

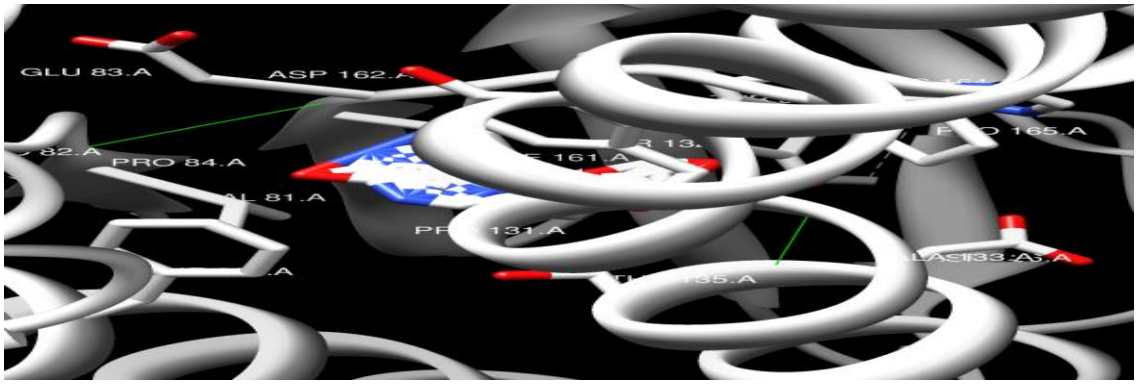


Fig. 5: Interaction of drugs against the protein 1KI3 using LAMARCKIAN GENETIC ALGORITHM (autodock). The thin dotted lines with colour ● represent interacting hydrogen bonds between the drug and the protein

DISCUSSION

Herpes Simplex Virus type 1 and type 2 (HSV-1 and HSV-2) are two members of Herpesviridae family, which infect almost 85% of the world population [19]. There is no method to eradicate herpes virus from the body, but anti-viral medications can reduce the frequency duration and severity of outbreaks [20].The current research paper is focussed on docking study of typical anti-herpes drugs with the thymidine kinase of herpes virus (PDB ID: 1KI3). On the basis of the binding energy values of the drugs (under study) obtained using the two different docking protocols, Ganciclovir is found to be the most potent drug.Ganciclovir [9-(1, 3 dihydroxy-2- propoxymethyl) guanine] is an acyclic analogue of guanosine that differs from acyclovir in having an additional hydroxymethyl group on the acyclic side chain. Viral DNA is inhibited by Ganciclovir [21]. It is available in oral and parenteral formulations. Oral Ganciclovir is poorly absorbed, with a bioavailability of only 5%. Intravitreal Ganciclovir implants are also available, with minimal systemic absorption. Ganciclovir undergoes triphosphorylation to become active, with the initial mono phosphorylation catalyzed by UL97-encoded kinase and subsequently by cellular kinases. Ganciclovir triphosphate inhibits viral DNA synthesis through competitive incorporation during viral DNA synthesis, thereby leading to DNA chain termination. In vitro, it is 10 times more potent than acyclovir against cytomegalovirus (CMV) and Epstein-Barr virus (EBV) and is just as effective as acyclovir against HSV-1, HSV-2 and Varicella zoster virus (VZV) [22]. The half life of ganciclovir is 3-4 h. Ganciclovir is currently used in the clinic to treat and prevent human CMV (HCMV) disease. Although it has marked antiviral activity against HSV in vitro and in vivo, Ganciclovir is not routinely used for the treatment of HSV infections. Yet, several reports have demonstrated the inhibitory effects of Ganciclovir on the growth of tumors in mice or rats inoculated in the HSV-1 TK gene-producing packaging cells [22]. In fact, Ganciclovir is currently envisaged in the combined gene therapy: chemotherapy of tumors transfected with the HSV-1 TK gene [23].

CONCLUSION

Docking study using two different docking protocols establishes Ganciclovir to be the most potent and efficient drug with the least binding energy of -201.66 Kcal/mol (using CDOCKER) and -9.10 Kcal/mol (using LAMARCKIAN GENETIC ALGORITHM) respectively amongst the five typical anti-herpes drugs (guanosine analogues) under study. Keeping the above study under consideration, further structural modifications can be carried out taking Ganciclovir as the reference of choice to develop newer congeners which can have better activity against herpes virus along with improved bioavailability.

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