Docking Studies of Adenosine Analogues with NS5 Methyltransferase of Yellow Fever Virus

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Abstract Yellow fever virus (YFV) is caused by single stranded positive RNA virus called *Flavivirus*. Till now no specific antiviral agents are available for the treatment of YFV, and despite a commercial YFV vaccine, there are still approximately 30,000 deaths worldwide each year and cases have been increasing in the last 20 years. Here, the effects of adenosine analogues and commercially available adenosine derivative drugs on NS5 methyltransferase of YFV have been performed by the comparative docking study. Based on the docking score, the glide energy and the number of interactions of the adenosine analogues with the Pubchem ID 13792 and 1077 showed the better scoring function than the best ranked commercially available adenosine analogue derived antiviral drug Cc3ado. From the docking result it reveals that these adenosine analogues can bind to the active site of NS5 methyltransferase protein and inhibit the viral replication.

Keywords NS5 · Adenosine analogue · Yellow fever virus · Flavivirus

Introduction

Yellow fever (YF) is a life-threatening mosquito-borne flaviviral hemorrhagic fever (VHF) characterized by severe hepatitis, renal failure, hemorrhage, and rapid terminal events with shock and multi-organ failure [1]. It is an acute

viral hemorrhagic disease caused by RNA virus with positive sense of the Flaviviridae family. YF virus is the prototype virus with a genome of 10,862 nucleotides with a 5' CAP structure and a nonpolyadenylated 3' end encoding a polyprotein of 3,411 amino acids which is cleaved by proteolytic processing to give rise to 11 viral polypeptides. Nucleotide sequence analyses of flavivirus genomes have led to new insights into genome structure and replication [2]. NS5 methyltransferase is also one of the important targets for anti-flaviviral drug discovery [3–5].

Till now there are no specific antiviral agents for the treatment of YF virus (YFV), and despite a commercial YFV vaccine, there are still approximately 30,000 deaths worldwide each year and cases have been increasing in the last 20 years [6, 7]. The virus is endemic in Africa and South America, but cases of YFV have been reported in non-endemic areas also [8, 9]. YFV is related to hepatitis C, dengue, West Nile and other viruses of human concern. Mosquito species of *Aedes* and *Haemagogus* transmit YFV and serve as a reservoir for the virus; humans and monkeys are the primary hosts for viral infection [6]. The disease may be limited to a mild febrile illness or may be more severe, including jaundice, renal failure, vascular instability and shock [6]. There is an approximately 50% case fatality rate in severe YFV cases [7].

The plus-strand RNA genome of flavivirus contains a 5' terminal cap 1 structure (m⁷GpppAmG). The flaviviruses encode one methyltransferase, located at the N-terminal portion of the NS5 protein, to catalyze both guanine N-7 and ribose 2'-OH methylations during viral cap formation. Representative flavivirus methyltransferases from dengue, YV, and West Nile virus (WNV) sequentially generate GpppA → m⁷GpppA → m⁷GpppAm. The 2'-O methylation can be uncoupled from the N-7 methylation, since m⁷GpppA-RNA can be readily methylated to m⁷GpppAm-

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RNA. N-7 methylation activity is essential for the WNV life cycle and, thus, methyltransferase represents a novel target for flavivirus therapy [4].

In the present study comparative docking study of adenosine analogues and commercially available adenosine derivative drug was performed to find the better inhibitor of NS5 methyltransferase protein of YFV to control this virus replication.

Materials and Methods

All computational analysis were carried out on Red Hat 5.1 Linux platform in Lenovo Intel core 2 duo processor 1 GB RAM.

Preparation of Adenosine Analogues

The coordinates of six adenosine analogues were obtained from the Pubchem (<http://pubchem.ncbi.nlm.nih.gov/>) (Fig. 1). The dataset of six adenosine analogue derived used a reference ligands (Fig. 2) are commercially available antiviral drug which were taken from one publication [10]. Each structure was assigned an appropriate bond order using the LigPrep script shipped by Schrodinger. The analogues were converted to.mae file format (Maestro, Schrodinger, Inc.) and optimized by means of the OPLS-

2005 (Optimized Potentials for Liquid Simulations) force field using default settings [11].

Preparation of Protein Target Structure

The starting coordinates of the NS5 methyltransferase [PDB ID: 3EVA] was taken from the Protein Data Bank (www.rcsb.org) and further modified for Glide docking calculations. For Glide (Schrodinger) calculations, NS5 complex was imported to Maestro (Schrodinger), the co-crystallized ligand was identified and removed from the structure and the protein was minimized using the Protein Preparation Wizard by applying an OPLS-2005 force field. Progressively weaker restraints were applied to non-hydrogen atoms only. This refinement procedure was done based on the recommendations by Schrodinger software, because Glide uses the full OPLS-2005 force field at an intermediate docking stage and is claimed to be more sensitive to geometrical details than other docking tools. Water molecules which are 5 Å away from the active site were removed and H atoms were added to the structure. The most likely positions of hydroxyl and thiol hydrogen atoms, protonation states and tautomers of His residues, and Chi 'flip' assignments for Asn, Gln and His residues were selected. Minimizations were performed until the average root mean square deviation of the non hydrogen atoms reached 0.3 Å.

Fig. 1 Structure of adenosine analogues with their Pubchem ID

Fig. 2 Structure of adenosine analogue derived commercially available antiviral drugs

Glide Docking and Scoring Function

Glide calculations were performed with Impact version v18007 (Schrodinger, Inc.) [12–14]. It performs grid-based ligand docking with energetics and searches for favourable interactions between one or more typically small ligand molecules and a typically larger receptor molecule, usually a protein [12]. Schrodinger recommends the performance of test calculations with different scaling factors for the van der Waal radii of the receptor and ligand atom, because steric repulsive interactions might otherwise be overemphasized, leading to rejection of overall correct binding modes of active compounds. After ensuring that the protein and ligands were in the correct form for docking, the receptor-grid files were generated using a grid-receptor generation program. To soften the potential for non-polar parts of the receptor, we scaled van der Waal radii of receptor atoms by 1.00 Å with a partial atomic charge of 0.25. A grid box with coordinates $X = 3.4860$, $Y = -36.804$ and $Z = 22.3858$ was generated at the centroid of the active site consisting of residues ILE132, ASP131, TRP87, SER56, ASP146, GLU111 and the size of ligands to be docked was selected from the workspace. The ligands were docked with the active site using the 'extra precision' Glide algorithm. Glide generates conformations internally and passes these through a series of filters. The first places the ligand centre at various grid positions of a 1 Å grid and rotates it around the three Euler angles. At this stage, crude score values and geometrical filters weed out unlikely

binding modes. The next filter stage involves a grid-based force field evaluation and refinement of docking solutions including torsional and rigid body movements of the ligand. The OPLS-2005 force field is used for this purpose. A small number of surviving docking solutions can then be subjected to a Monte Carlo procedure to try and minimize the energy score. The final energy evaluation is done with Glide score and a single best pose is generated as the output for a particular ligand.

$$\text{GScore} = a * \text{vdW} + b * \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

where, vdW is van der Waal energy, Coul is Coulomb energy, Lipo is lipophilic contact term, HBond is hydrogen-bonding term, Metal is metal-binding term, BuryP is penalty for buried polar groups, RotB is penalty for freezing rotatable bonds, Site is polar interactions at the active site, and the coefficients of vdW and Coul are: $a = 0.065$, $b = 0.130$.

ADME Screening

The QikProp program [15] was used to obtain the ADME properties of the analogues. It predicts both physically significant descriptors and pharmaceutically relevant properties. All the analogues were neutralized before being used by QikProp. The neutralizing step is essential, as QikProp is unable to neutralize a structure and no

Fig. 3 The binding site of NS5 methyltransferase of YFV bound with the substrate

properties will be generated in the normal mode. The program was processed in normal mode, and predicted 44 properties for the molecules, consisting of principal descriptors and physiochemical properties with a detailed analysis of the log P (octanol/water), QP%, and log HERG. It also evaluates the acceptability of the analogues based on Lipinski's rule of 5 [16], which are essential for rational drug design.

Results and Discussion

Binding Models of Co-crystallized Compound with NS5 Methyltransferase of YFV

The X-ray structure of YFV (PDB 3EVA) was selected as the starting reference for molecular docking analysis [17]. For a validation of the accuracy of the docking program glide XP approach was used in this study, the RMS

deviation between the crystal structure (PDB 3EVA) and the most reasonable binding modes of co crystallized ligand docked with glide XP was calculated. It forms six H-bond with the receptor and binds to the amino acid Ser56, Trp87, Glu111, Asp131, Ile132 and Asp146. The results of control docking showed that Glide XP determined the optimal orientation of the docked inhibitor, co-crystallized ligand to be close to that of the original orientation found in the crystal (Fig. 3). The RMS deviation between the experimental docked conformation and the calculated docked conformation for co crystallized ligand in RdRp of YF was 0.180 Å.

Docking of Adenosine Analogues

To study the molecular basis of interaction and affinity of binding of adenosine analogues, all the ligands were docked into the active site of NS5 methyltransferase. Commercially available six antiviral drugs derived from adenosine analogue were studied as a reference ligand. The docking result of these adenosine analogues is given in Table 1 and reference ligand in Table 2. The ranking of ligands was based on the glide score. Superposition of the docked adenosine analogues in 3EVA revealed that the binding mode of these inhibitors in NS5 methyltransferase is considered to be essentially similar and bind in the same orientation and similar position in terms of the common structure. The result demonstrates that docking simulation can dock all the adenosine analogues into the same binding site as well (Fig. 4). As these molecules have the same backbone structure, it is obvious that they bind in a similar pattern in the active site of NS5 methyltransferase. Comparing the docking score, glide energy and number of interaction, it was found that the adenosine analogues (Pubchem ID 13792 and 1077) shows better scoring function than best ranked commercially available drug Cc3ado. The interaction of best ranked adenosine analogue and commercially available adenosine analogue derivative antiviral drug are shown in Fig. 5. This proved that adenosine analogues (Pubchem ID 13792 and 1077) could be potential drugs for second-generation drug development.

Table 1 Docking score of adenosine analogues

Sl. no.	Pubchem ID	Glide score	Glide energy (kcal/mol)	No. of H-bond	Residues
1	13792	−12.696	−60.913196	9	ILE132, ARG84, ASP131, GLU111, SER56, GLY56, GLY86, GLY85
2	1077	−12.619	−66.517	6	ARG84, GYL85, GLY86, HIS110, ILE132, ASP131
3	107784	−10.984435	−60.611678	8	ASP146, TRP87, SER56, GLY86, GLU111, ILE132, ASP132
4	1763	−9.615846	−49.080106	6	ASP131, ILE132, HIS110, GLY86, ARG84, SER56
5	99302	−8.369612	−51.802791	4	GLU111, HIS110, ILE132, ASP131
6	126125	−8.456823	−57.282651	5	GLU111, HIS110, ILE132, ASP131

Table 2 Docking score of commercially available antiviral drug derived from adenosine analogue

Sl. no.	Reference ligand	Glide score	Glide energy (kcal/mol)	No. of H-bond	Residues
1	Cc3ado	−9.173567	−43.827243	5	GLU111, ILE132, ASP131, ASP146
2	Ribavirin	−8.333843	−46.108451	8	ASP146, GLU111, ARG84, SER56, GLY86
3	Cidofovir	−8.146033	−51.785644	8	SER56, GLY86, ARG84, GLY85, HIS110, ASP146
4	Mycophenolic acid	−7.967805	−47.998307	3	SER56, ARG84, THR104
5	Viramidine	−6.920761	−38.608061	6	GLY86, GLU111, CYS82, GLU81
6	Emtricitabine	−5.690171	−34.273233	2	THR104, ASP131

Fig. 4 Superimposition of adenosine analogue to the active site of NS5 methyltransferase protein of YFV

Predicted ADME Properties

We analysed 44 physically significant descriptors and pharmaceutically relevant properties of adenosine

analogues, among which were molecular weight, H-bond donors, H-bond acceptors, log P (octanol/water), log P MDCK, log Kp (skin permeability), humoral absorption and their position according to Lipinski's rule of 5

Fig. 5 Interaction of best ranked adenosine analogue (a) and best ranked adenosine analogue derived commercially available antiviral drug Cc3ado with NS5 methyltransferase of YFV

Table 3 Principal descriptors calculated for adenosine analogues by Qikprop simulation

Ligand no.	Molecular weight	Molecular volume	VDW PSA	HB donors	HB acceptors	Rotatable bonds
1763	326.373	701.551	14.095	9.500	5.500	1
13792	384.409	907.240	14.095	10.833	7.833	2
107784	413.4719	–	–	–	–	–
99302	297.331	648.805	21.358	7.500	6.500	1
1077	398.414	–	–	4	10	7
126125	343.375	807.335	57.471	7.500	6.500	1

Table 4 Physiochemical descriptors calculated for adenosine analogues by Qikprop simulation

Ligand no.	QP logPo/w	QP (%)	Log HERG	QPP Caco	QPP MDCK	Rule of 5
1763	–1.153	46.524	–2.037	156.716	79.722	1
13792	–1.143	9.785	–2.219	7.296	3.683	2
5136	–	–	–	–	–	–
99302	19.119	59.283	–1.693	584.806	362.673	1
1077	–	–	–	–	–	–
126125	19.408	63.695	–2.344	584.806	571.933	1

QP logPo/w QP log P for octanol/water, *QP %* % human oral absorption in GI ($\pm 20\%$) (<25% is poor), *log HERG* HERG K⁺ channel blockage (concern below –5), *QPP Caco* apparent Caco-2 permeability (nm/s) (<25 poor, >500 great), *QPP MDCK* apparent MDCK permeability (nm/s) (<25 poor, >500 great), *Rule of 5* Lipinski rule of 5 violations

(Tables 3, 4). Lipinski's rule of 5 is a rule of thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including its ADME. However, the rule does not predict if a compound is pharmacologically active. In this study, all the 6 ligands shows allowed values for the properties analysed and exhibited drug-like characteristics based on Lipinski's rule of 5.

Conclusion

The availability of NMR and X-ray crystallographic structures of NS5 methyltransferase complexes represents an enormous advantage in the fields of structural biology and medicinal chemistry. Nevertheless, the new computational tools can be successfully employed for the verification of the interactions emerged from crystal structures, because they combine conformational search procedures with a scoring function. In this study, comparative docking study was carried out with the selected six adenosine analogues and commercially available antiviral drugs which is a derivative of adenosine analogue. Based on the docking score, glide energy and number of hydrogen bonding it was found that the adenosine analogue with Pubchem ID 13792 and 1077 shows the better scoring

function than the best ranked commercially available antiviral drug Cc3ado. From the result it is concluded that these adenosine analogue can be potential drug against YFV.

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