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Molecular docking revealed the binding of nucleotide/side inhibitors to Zika viral polymerase solved structures

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ABSTRACT
A new Zika virus (ZIKV) outbreak started in 2015. According to the World Health Organization, 84 countries confirmed ZIKV infection. RNA-dependent RNA polymerase (RdRp) was an appealing target for drug designers during the last two decades. Through molecular docking, we screened 16 nucleotide/side inhibitors against ZIKV RdRp. While the mode of interaction with ZIKV is different from that in the hepatitis C virus (HCV), nucleotide/side inhibitors in this study (mostly anti-HCV) showed promising binding affinities (−6.2 to −9.7 kcal/mol calculated by AutoDock Vina) to ZIKV RdRp. Setrobuvir, YAK and, to a lesser extent, IDX-184 reveal promising results compared to other inhibitors in terms of binding ZIKV RdRp. These candidates would be powerful anti-ZIKV drugs.

Introduction
The Zika virus (ZIKV) was first identified in Uganda in 1947 [1,2]. The viral infection emerged again in 2007 in African countries such as Nigeria, Senegal and Gabon [3]. Starting from 2015 a new, rapid, ZIKV outbreak emerged in Latin America [4]. At the time of writing the infection has been recorded in 84 countries worldwide [5]. The World Health Organization in February 2016 flagged ZIKV as a public health emergency of international concern [6]. Aedes mosquitoes are responsible for the spread of ZIKV. These include Aedes africanus, Aedes aegypti, Aedes luteocephalus and Aedes albopictus [7–10]. ZIKV infection is clinically detected easily as the virus can be found in any body fluids like blood, urine and even saliva [6,11]. Mild symptoms characterize ZIKV infection such as slight fever, rash, arthralgia and conjunctivitis [5,9,10]. In 2016, Mlakar and co-workers confirmed the link between microcephaly developed in newborns and mothers infected by ZIKV during pregnancy [12]. Additionally, severe neurological diseases and sterility are reported [13].

ZIKV is a member of the Flavivirus genus characterized by a single-stranded RNA genome that encodes a polyprotein of 3400 amino acids. The polyprotein is processed by viral and host cell proteases into 10 structural and non-structural proteins [3,6]. Non-structural 5 (NS5)
is the polymerase enzyme responsible for the formation of new copies of the viral RNA [3,14–16]. ZIKV NS5 has two domains: RNA methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp) [17]. RdRp is an enzyme vital for the virus’ life cycle and it is evolutionarily highly conserved [18]. RdRp harnesses the free nucleotides in the host cell cytoplasm to build a complementary RNA strand of the viral genetic material [17,19–22]. Blocking RdRp active sites ceases new virion generation and hence eradicates the viral infection [23,24]. RdRp have three subdomains: fingers, thumb and palm. The palm subdomain has the consecutive, highly conserved, aspartates that represent the active site [18,25–28]. The palm subdomain has five motifs (A to E). The consecutive aspartates are protruded from the beta-turn of motif C [21]. The palm motif is the most conserved part of RdRp since they possess the catalytic amino acids that transfer nucleotides to the primer RNA [21]. During the last two decades, hepatitis C virus (HCV) and human immunodeficiency virus (HIV) research has revealed a lot of new compounds that mimic the nucleotides but block the function of the polymerase and reverse transcriptase in HCV and HIV, respectively. The purpose of this study is to screen some of these nucleotide/side inhibitors against the ZIKV polymerase solved structures and compare their binding energies and modes of interaction [29,30]. Recently, five anti-HCV drugs have been tested against ZIKV models built in silico [31]. The results revealed binding energies comparable to that of HCV and consequently possible inhibition of ZIKV polymerase. Moreover, ZIKV was inhibited in vitro and in vivo (human cell lines and rats infected by ZIKV) after treatment with sofosbuvir (an anti-HCV drug approved by the Food and Drugs Administration) [32–34].

In recent months, five experimentally solved structures were deposited in the Protein Data Bank (PDB) [13,17,35,36]. These structures are targeted in this study using molecular docking by 16 nucleotide/side inhibitors (mostly anti-HCV drugs). These inhibitors are listed in Figure S1 including some drugs that currently exist in the market: sofosbuvir (anti-HCV), tenofovir (anti-HIV) and ribavirin (wide-acting antiviral). Other inhibitors are still under clinical trials against HCV RdRp.

**Materials and methods**

**Structural retrieval and preparation**

Experimentally solved structures of ZIKV NS5 polymerase were retrieved from the Protein Data Bank [37]. Five structures were downloaded (PDB ID codes: 5TFR, 5TIT, 5TMH, 5U04 and 5WZ3). The first and third PDBs were for full-length NS5 while the rest were for the RdRp domain alone. Only the RdRp domain was kept for this study while other domains and ligands were removed from the PDB files. Water and metal ions were maintained in the PDB files since they are crucial in mediating the interaction between nucleotides and the active site amino acids in polymerases. Missing hydrogen atoms were added to the X-ray crystallography solved structures. ZIKV polymerase models used in previous studies were compared to the solved structures [31]. The models were built using the threading comparative modelling server Phyre 2, using Dengue virus RdRp (PDB ID 2j7u) as a template, then validated using the Structure Analysis and Verification Server (SAVES) [38–40]. The HCV RdRp solved structure (PDB ID: 2XI3) was used as a control for comparison (after ligand removal and hydrogen atom addition). Structural alignment carried out using VMD software to detect differences and conservation of the structural elements [41,42].
**Molecular docking**

The binding of 16 ligands was assessed against ZIKV RdRp. These compounds have been studied during the last two decades. They included anti-HCV and anti-HIV nucleotide/side inhibitors (sofosbuvir, IDX-184, MK0608, mericitabine, ribavirin, 2′-C-methylcytidine, valopicabine, uprifosbuvir, tenofovir, setrobuvir, R1479, PSI_6206, PSI_6130, YAK, BMS_986094 and balapiravir) [23, 43–45] (see Figure S1). Sofosbuvir is approved by the FDA against HCV while tenofovir is an approved anti-HIV drug [23,24,27,46]. Before starting the docking study, nucleotide inhibitors were modified to be in their phosphorylated active form to resemble physiological conditions. Geometry optimization was then quantum mechanically done using density functional theory (DFT) (B3LYP functional) [47,48] for the compounds to be in their lowest energy state. AutoDock Vina software was used to perform molecular docking [49]. ZIKV RdRp and the ligands were treated as rigid and flexible, respectively. A grid box of 30 × 30 × 30 was used in all of the docking calculations, with the centre set between the two active site aspartates (ASP665 and ASP666 in ZIKV, and ASP318 and ASP319 in HCV).

Binding affinity values (in kcal/mol) were retrieved from the output files to compare ZIKV RdRp complexes to that of HCV. AutoDock Tools (ADT) [50], PyMOL [51] and Maestro software [52] were used to examine the docking poses.

**Results**

**Structural alignment**

The robust sequence, hence structural, conservation among the five recently solved structures of ZIKV RdRp (first release in the Protein Data Bank was 12 October 2016) is clearly shown in Figure 1(a). Among the five structures, the sequence identity is at least 76.16%. Notably, the active site aspartates ASP665 and ASP666 (represented by yellow licorice in Figure 1(a)) have conserved orientation in space. Even the loops and turns are structurally conserved. The less conserved amino acids (represented by a red cartoon) are apart from the active site. On the other hand, HCV RdRp shows sequence and structural differences compared to ZIKV RdRp (Figure 1(b)). Despite the lower percentage identity (11.81%), HCV and ZIKV RdRps have a highly conserved active site. The differences (red cartoon) appear mainly in the fingers and thumb domains aside from the active site located in the palm domain. Figure 1(c) demonstrates the superposition of the ZIKV solved structure (PDB ID: 5TFR) and ZIKV model built by the Phyre 2 server with a sequence identity of 97.43%.

**Molecular docking**

Molecular docking tests the binding efficiency of the studied ligands to the ZIKV RdRp active site. It also enables us to compare between the ZIKV and HCV binding modes. Figure 2 represents the average binding affinity (in kcal/mol) calculated by AutoDock Vina for the five solved ZIKV RdRp structures (red column), ZIKV RdRp models (green column) and the HCV RdRp solved structure (PDB ID 2XI3) (blue line). Error bars represent the standard deviation from the mean.

We now examine the docked structures to find the differences in the binding of ligands to the HCV and ZIKV structures. Table 1 summarizes the number of H-bonds formed between the ligands and the RdRps of HCV (PDB ID: 2XI3) and ZIKV (PDB ID: 5TFR). The amino acids...
Figure 1. (a) The superposition of the five solved structures of ZIKV RdRp (PDB ID 5TFR, 5STT, 5TMH, 5U04 and 5WZ3). The backbones are represented by cartoon coloured according to conservation using VMD software. Blue coloured ribbons for highly conserved and red for the least conserved amino acids. (b) Superposition of the solved structure of ZIKV RdRp (PDB ID 5TFR) and HCV RdRp (PDB ID 2X13) represented as A. The active site aspartates are represented by yellow licorice. (c) ZIKV model built by PHYRE2 server in a previous study using dengue virus RdRp (PDB ID 2J7U) as a template. The representation is as that of A and B while active site aspartates (ASP665 and ASP666) are labelled.

Figure 2. Average binding affinity values calculated by AutoDock vina software for the docking of 16 nucleotide/side inhibitors into ZIKV RdRp models (green column) and solved structures (red column) and HCV RdRp solved structure (blue line). Error bars represent standard deviations of the means.
involved in H-bond formation are also listed. The water molecules near the active site pockets are involved in H-bonding with some ligands. The numbers of water molecules mediated H-bonding with ligand are also listed in Table 1.

The best three nucleotide/side inhibitors against ZIKV RdRp, based on the binding affinity values, were setrobuvir, YAK and IDX-184. Figure 3(b) is a 2D interaction diagram of the docking of the three best compounds for ZIKV RdRp (setrobuvir, YAK and IDX-184). Amino acids are represented by circles coloured according to the scheme in the bottom of the figure.

**Discussion**

Viral RNA was used as a template to construct a new copy of the viral genetic material through the free nucleotides that exist in the host cell cytoplasm. This is a process manipulated by
RdRp. This enzyme has a conserved fold that is found in almost all polymerases. Three distinct subdomains characterize any RdRp: fingers, thumb and palm. The catalytic, consecutive, aspartates found in the palm subdomain of RdRp are responsible for the polymerization
This suggests possible inhibition if a suitable ligand can target and effectively bind to ZIKV RdRp.

From the structural conservation (Figure 1(a,b)), it is suggested that good binders (molecules that are well fitted in the active site cavity and that interact with the active site amino acids through H-bonding and metal interactions) to HCV RdRp may possess considerable binding affinity to ZIKV RdRp. This was tested in previous studies [53] using ZIKV models built in silico (before any solved ZIKV RdRp was released in the Protein Data Bank) and confirmed experimentally on sofosbuvir [31,32]. Figure 1(c) shows the strong structural conservation between ZIKV model and solved structure (PDB ID 5TFR). The root mean square deviation (RMSD) for fitting all atoms is 2.1 Å while fitting all alpha carbons RMSD is 0.82 Å. This confirms the quality of ZIKV RdRp models built in silico by the Phyre 2 server in a previous study [41].

We have performed a molecular dynamics simulation (MDS) in a previous study on the ZIKV NS5 model for 444 ns [41]. It was concluded that the binding affinities of some nucleotide inhibitors were not affected by the protein dynamics. All the ligands can fit inside the ZIKV NS5 active site cavity during the entire period of the MDS. Binding affinity values for all the ligands used in the docking study are depicted in Figure S2. All the values lie between −9.1 and −5.6 kcal/mol, which means good binding energies.

Figure 2 shows that the binding affinities to ZIKV RdRp of all the nucleotide/side inhibitors are comparable to that of HCV RdRp. The average binding affinity values for the HCV RdRp structure (PDB ID: 2XI3 blue line) are slightly more negative (better) compared to ZIKV RdRp solved structures (red column) and models (green column). Fortunately, the differences in the binding energies are not significant. This means that the ligands are able to bind to the ZIKV active site with good binding affinity values (>−6.5 kcal/mol). Interestingly, setrobuvir, YAK and, to a lesser extent, IDX-184 are significantly better, in their binding affinities for all RdRps, compared to the other compounds. These results support the previous studies made on HCV, ZIKV and human coronavirus RdRps models built in silico [23,28,31].

The binding modes of ligands to both HCV and ZIKV RdRps were examined. For each ligand, both the number of H-bonds formed and the amino acids involved in H-bond formation are totally different (see Table 1). The most frequent amino acids that are involved in H-bonding with ligands in HCV RdRp are the active site aspartate (ASP318) and ARG158. Water molecules near the active site form H-bonds to both the ligands and the polymerase. This facilitates binding, besides the metal interaction through the two Mg$^{2+}$. On the other hand, for ZIKV RdRp the most frequently amino acids that are involved in H-bonds are the active site aspartates (ASP665 and ASP666) and SER663. Rare cases of water molecules forming H-bonds with ligands have been reported in ZIKV RdRp (Table 1). In addition, no divalent metal ions exist near the active site (Figure 3(a)).

Metal coordination bonds with the sugar oxygen formed in HCV RdRp. This coordination bond is not present in the ZIKV RdRp. Interestingly, all five solved ZIKV structures have two Zn$^{2+}$ lying apart from the active site. Figure 3(a) illustrates the position of these two metal ions (red circles) for the Sftr structure. The metal ions are in the fingers and thumb domains, apart from the two catalytic aspartates (Asp665 and Asp666 represented by yellow sticks).

In Figure 3(b), the consecutive active site aspartates (the red circles representing Asp665 and Asp666) are located around the methyl sulphonamide groups of setrobuvir and YAK. On the other hand, these aspartates form H-bonds to sugar and guanosine moieties of IDX-184. YAK also mediates its binding through H-bond formation with SER603 and SER663.
Setrobuvir hydrophobic moieties of fluorophenyl and methyl quinoline are surrounded by three hydrophobic amino acids (green circles): ILE475, TYR477 and TRP797. This pattern persists for the fluorophenyl and pyrindin groups of YAK (PHE400, ILE475, TYR477, VAL606). On the other hand, TYR609 and ILE799 are lining the methyl group found in the 2′carbon of the ribose of IDX-184. This adds to the stability of these three compounds in the active site cavity of RdRps compared to the others.

In HCV, four H-bonds are formed between IDX-184 and ARG48, ARG158, ASP225 and ASP318 and two H-bonds with water molecules. On the other hand, only two H-bonds are formed in the case of ZIKV (ASP665 and ASP666) (see Table 1). Consequently, IDX-184 docked slightly better to HCV RdRp (−9.1 kcal/mol) compared to ZIKV RdRp (−8.4 kcal/mol). This is mainly due to the metal interactions that add to the stability of the complex formed in the case of HCV RdRp.

Conclusions

ZIKV RdRp is an enzyme vital for the ZIKV life cycle. Targeting RdRp has been reported for many viruses. The anti-HCV RdRp drug, sofosbuvir, has previously been tested against ZIKV RdRp. The first five solved structures of ZIKV (PDB ID: 5TFR, 5TIT, 5TMH, 5U04 and 5WZ3) were used in this study to confirm previous reports by utilizing sixteen different nucleotide/side inhibitors developed in the past two decades against HCV and HIV. Three compounds show better binding affinity to ZIKV RdRp, namely setrobuvir, YAK and IDX-184. These compounds interact with the active site pocket with H-bonds and hydrophobic interactions. Overall, the nucleotide/side inhibitors used in this study show slightly lower binding affinity to ZIKV RdRp solved structures compared to that for the HCV RdRp solved structure (PDB ID 2XI3). Examining the docked structures revealed, in the case of ZIKV RdRps, the lack of metal interactions. Additional studies (including the dynamics of the protein) will be performed to test the reliability of the binding and to suggest compounds that are more potent against ZIKV RdRp.

References


