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Identification of novel VP35 inhibitors: Virtual screening driven new scaffolds

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ABSTRACT

Ebola virus is a single-stranded, negative-sense RNA virus that causes acute and serious life-threatening illness. In recent years the Ebola virus has spread through several countries in Africa, highlighting the need to develop new treatments for this disease and boosting a new research effort on this subject. However, so far there is no valid treatment for disease created by this pathogen. The Ebola virus Viral Protein 35 (VP35) is a multifunctional protein which is critical for virus replication and infection, and it is considered as a future target for drug development. In this study, we collected 144 VP35 inhibitors which shared the same core scaffold, and a common feature pharmacophore model HypoA was built based on inhibitor-receptor complexes. All 141 compounds were aligned based on the common feature pharmacophore model HypoA (three compounds could not map onto HypoA). The pharmacophore model HypoA was further optimized according to the actual interactions between inhibitors and VP35 protein, resulting in a new pharmacophore model HypoB which was applied for virtual screening. A 3D QSAR model was established by applying the 141 aligned compounds. For the training set, the 3D QSAR model gave a correlation coefficient r^2 of 0.897, for the test set, the correlation coefficient r^2 was 0.757. Then a virtual screening was carried out, which comprehensively employing the common feature pharmacophore model, 3D QSAR model and docking study, their combination in a hybrid protocol could help to mutually compensate for their limitations and capitalized on their mutual strengths. After the above three virtual screening methods orderly filtering, seven potential inhibitors with novel scaffolds were identified as new VP35 inhibitors. The mapping results of hit compounds onto pharmacophore model and 3D QSAR model, and the molecular interactions of the potential inhibitors with the active site residues have been discussed in detail.

1. Introduction

Ebola virus disease (EVD) first outbroke in the Democratic Republic of Congo in 1976. Since 1976 there have been several outbreaks, the recent 2013–2015 epidemic in Western countries of the African, including Guinea, Liberia, and Sierra Leone, is believed to be one of the massive eruptions in recorded history [1]. Ebola virus infection is characterized by severe hemorrhagic fever with a case fatality of up to 90% in some outbreaks [2]. As of June 10, 2016, the World Health Organization has reported that a total of 28,616 Ebola cases have been found in Guinea, Liberia and Sierra Leone, with 11,310 deaths. Although treatment centers provide supportive care, EVD epidemic is challenging since the widespread nature of the current EVD outbreak as well as cultural practices in the affected countries. In addition, no effective therapeutic agents are available for EVD-endemic countries [3]. The large number of cases and the high mortality rate, combined with the lack of effective Food and Drug Administration approved treatments, necessitate the development of potent and safe therapy to combat the current and future outbreaks.

Ebola virus encodes seven polypeptides from its RNA genome, including glycoprotein (GP), nucleoprotein (NP), RNA-dependent RNA polymerase (L), VP35, VP30, VP40 and VP24 [4,5]. Out of these proteins, we have focused on developing novel VP35 inhibitors by using computer-aided drug design method since there have been

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reported more than one hundred small molecular VP35 inhibitors [6], and the crystal structures of inhibitor-VP35 complex were reported [6], which provide material basis for development of novel VP35 inhibitors by using virtual screening method. Multifunctional VP35 acts as a component of the viral RNA polymerase complex, as a structural/assembly factor, and as a suppressor of host IFN responses [7–9]. VP35 inhibits phosphorylation, activation, and nuclear localization of IRF-3 [10–13] and inhibits activation of the cellular antiviral kinase RNA-dependent protein kinase (PKR) [14]. VP35 also regulates viral RNA synthesis [15,16] and plays important role in RNA silencing suppression [17]. Therefore, a functional VP35 is required for efficient viral replication and pathogenesis; knockdown of VP35 leads to inhibit viral amplification and protects against lethal infection in mice [18]. In summary, Ebola virus VP35 protein is a potential drug target for development of drugs to combat EVD.

In this investigation, we collected a total of 144 small molecular inhibitors (see table S1) of VP35 and nine crystal structures of inhibitor-VP35 complex. Based on these data, virtual screening for identifying new VP35 inhibitors was performed. Firstly, we established a common feature pharmacophore model HypoA based on the active conformation of one inhibitor derived from the crystal structure of inhibitor-VP35 complex (PDB code: 4IBC) because that the 144 inhibitors shared the same core scaffold. Then, the collected 144 compounds were aligned based on the common feature pharmacophore model HypoA. The pharmacophore model HypoA was further optimized according to the actual interactions between inhibitors and VP35 protein, resulting in a new pharmacophore model HypoB which was applied for virtual screening. A 3D QSAR model was built by using the aligned compounds. A hybrid virtual screening method was used for identifying new VP35 inhibitors, including pharmacophore-based (PB-VS) virtual screening, 3D QSAR-based (QSARB-VS) virtual screening and docking study. In the three virtual screening methods, PB-VS is fast and simple, therefore, it was used firstly in the hybrid virtual screening. However, the pharmacophore model HypoB used for virtual screening is a common feature pharmacophore model of the core scaffold which has not the ability of considering the structure-activity relationship of the 144 inhibitors. Therefore, the successive virtual screening method was QSARB-VS which could predict the activities of the hit compounds. The last virtual screening method was docking study which is the most complexity, but was applied only to a small subset of the entire database. Finally, seven potential active compounds were selected and would be purchased for activity tests. And the hit compounds have been discussed in details, including pharmacophore mappings, 3D QSAR model mappings and docking study.

2. Materials and methods

2.1. Pharmacophore modeling, pharmacophore based alignment and PB-VS

The "Feature Mapping" protocol in the Discovery Studio 3.1 (Accelrys Inc., San Diego, CA, USA) was applied for pharmacophore modeling. The common feature pharmacophore model HypoA of VP35 inhibitors in this study was established based on the active conformation of one inhibitor GA246 complexed with VP35 protein. HypoA was applied for alignment of the 144 inhibitors by using the protocol of "Ligand Pharmacophore Mapping" in Discovery Studio 3.1. According to actual inhibtor-VP35 interactions, the pharmacophore HypoA was optimized, one hydrogen-bond acceptor feature and one hydrogen-bond donor were deleted, resulting in pharmacophore model HypoB. The established pharmacophore model HypoB was employed as a 3D structural query to screen commercially available chemical databases "Diversity Libraries" including 105,145,5 compounds (Life Chemicals Inc., Burlington, Canada), using the "Search 3D Database" protocol in Discovery Studio 3.1. The query was performed using the best search method. Only those compounds possessing fit value higher 3.0 were chosen.

2.2. 3D QSAR modeling and QSARB-VS

Among all the 141 aligned inhibitors (three compounds could not map onto the pharmacophore model HypoA), 80% (that is 113 compounds) were utilized as a training set for QSAR modeling. The remaining 20% (that is 28 compounds) were chosen as an external test subset for validating the reliability of the QSAR model. The training set compounds and test set compounds were generated by the "Generate Training and Test Data" protocol in Discovery Studio 3.1 using diverse molecules split method.

The inhibitory activity of the compound in literature [K_D (mol/L)] was initially changed into the minus logarithmic scale [pK_D (mol/L)] and then used for subsequent QSAR analysis as the response variable.

In Discovery Studio 3.1, the CHARMm force field is applied and the Van der Waals potential together with the electrostatic potential are treated as separate terms. As for the Van der Waals potential, a carbon atom with a radius of 1.73 Å is used as a probe. As the electrostatic potential probe, A + le point change is used while distance-dependent dielectric constant is used to mimic the solvent effect. A Partial Least-Squares (PLS) model is built using energy grids as descriptors. QSAR models were built by using the "Create 3D QSAR Model" protocol in Discovery Studio 3.1.

The QSARB-VS was carried out by employing the "Calculate Molecular Properties" protocol. The parameter "Molecular Properties" was set to the established 3D QSAR model. Only compounds owning the predicted pK_D value higher 3.5 were selected for the next docking study.

2.3. Molecular docking

All the docking studies were performed by GOLD 5.1. The crystal structure of VP35 protein (PDB code: 4IBC) were used in the docking study. All hydrogen atoms were added to the protein by using Discovery Studio 3.1 and the ChARMm force field was assigned. The binding site was defined as a sphere containing the residues that stay within 10 Å from the ligand, which is large enough to cover the ligand binding region at the active site. The scoring functions and docking parameters were optimized in advance by docking the nine inhibitors complexed with the VP35 protein back to the active site of their receptor.

3. Results and discussion

3.1. Data analysis

We collected a total of 144 VP35 inhibitors from the literature [6], which share the same 4-acetyl-3-hydroxy-1-phenyl-1H-pyrrol-2(5H)-one scaffold. All the 144 compounds were assigned estimated K_D values which were obtained by using single point titration data to fit corresponding equations (the detail description about this method can be found in the literature [6]), and some of them, 34 compounds were given the experimental activities which were tested by applying NMR. In fact, the method obtained the estimated binding constants (estimated K_D values) was a simplified titration study, which used I303 as an indicator of binding and carried out a single point titration with 80 μM small-molecule ligand, then the experiment data was fitted corresponding equations to obtain the K_D values. The determination coefficient (r^2 value) between K_D values obtained using single point data and