Combinatorial Drug Design Approach for the Discovery of Potential Inhibitors against Influenza Neuraminidase

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Abstract: The frequent emergence, in recent years, of strains of influenza virus, which are resistant to current treatment regimens poses a devastating problem to world health, particularly in developing nations such as South Africa. This necessitates the discovery and development of novel therapeutic agents. This study employs computational methods to identify compounds, which could exhibit potent inhibitory activity against neuraminidase, a crucial enzyme in Influenza virus, with a preferably greater inhibitory activity than a known neuraminidase inhibitor, Zanamivir. Molecular docking and virtual screening were employed to identify hits that were structurally similar to Zanamivir. Potential hits were retrieved from the DrugBank database for pharmacokinetic analysis using the online software admetSAR. Three compounds were identified after the virtual screening. Pharmacokinetic properties revealed that all three compounds adhered to the Lipinski rule of five and were structurally similar to Zanamivir. Molecular docking of all three compounds into the binding site of neuraminidase showed that compound DB03321 had better binding interactions when compared to the rest of the compounds. DB03321 formed a greater number of hydrogen bonds with the residues of the active site with the highest binding affinity of -7.9kcal/mol, when compared to other compounds obtained through screening. DB03321 showcased preferable interactions with binding site residues of neuraminidase relative to Zanamivir. These findings could serve as a step forward in the development of novel inhibitors of influenza neuraminidase, which could potentially act with greater potency than currently available therapeutic agents.

Keywords: Influenza Virus, Neuraminidase, Virtual Screening, Pharmacokinetics, Molecular Docking

Introduction

Influenza, also called flu, is an acute viral respiratory infection, transmitted by the influenza virus (Taubenberger and Morens, 2008; Krammer et al., 2018). Influenza is spread through air droplets, when infected persons cough, spit or sneeze (Fig. 1). It is characterized in its full form by the sudden onset of high fever, coryza, cough, headache, malaise and inflammation of the upper respiratory tract and trachea (Taubenberger and Morens, 2008; Krammer et al., 2018). Annually, 3-5 million people are infected and more than 200 000 people die from its infection worldwide (WHO, 2018). Over 90% of influenza deaths occur in low and middle-income countries (de Francisco et al., 2015). South Africa is one of the countries that have the burden of influenza, with about 6000-11000 deaths reported as a result of influenza annually (NACD, 2017).

The current treatment of influenza consists of oseltamivir (75mg) taken twice daily for 5 days, or 10mg zanamivir inhaled twice daily for 5 days (NACD, 2017). Previously, amantadine and rimantadine were also used to treat influenza, but their use is now discouraged due to widespread resistance (Hussain et al., 2017). These drugs mainly inhibit neuraminidase, sialidase (von Itzstein and Thomson, 2009) matrix protein 2 (M2) and viral fusion protein hemagglutinin (Li et al., 2015). However, inappropriate use of anti-influenza drugs may lead to the development of drug resistant strains.
Viral neuraminidase (Fig. 2) is a glycoside hydrolase enzyme found on the surface of influenza virus (Shtyrya et al., 2009). It allows for the release of the virus from the host cell and the splitting of sialic acid groups from glycoproteins while acting as a prerequisite for the replication of influenza virus (Shtyrya et al., 2009). Thus, it plays an important role in proliferation and survival of the virus. As such, therapeutic targeting of neuraminidase is a viable approach that has been harnessed over the years towards anti-influenza drug design. Therapeutic inhibition of neuraminidase arrest the release of the virus from an infected cell and thus help to prevent transmission (Krammer et al., 2018).

Neuraminidase inhibitors impede sialic acid cleavage, hence hindering host cell invasion by the influenza virus (Hata et al., 2008). Zanamivir was approved in 1999 as neuraminidase inhibitor, however, the development and transmission of drug-resistance to Zanamivir has necessitated the continuous search for new inhibitors that can overcome resistant strains of the virus (Hurt et al., 2009; Oxford, 2000). Thus, these new inhibitors will provide alternative for patients with drug-resistant influenza.

The focal points of drug discovery include identifying lead compounds that show pharmacological activity against a biological target and subsequently optimizing the potency and pharmacological effects of these compounds (Lionta et al., 2014). In this study, structure based methods, including virtual screening and molecular docking will be employed to search for novel
neuraminidase inhibitors that could possess enhanced therapeutic properties relative to Zanamivir and possibly overcome resistance. With the growing threat of resistant strains of the influenza virus, the discovery of new potent antiviral agents with fewer limitations and improved effectiveness would assist in the treatment of Influenza.

Computational Methodology

Retrieval and Preparation of X-Ray Crystal Structure of Influenza Neuraminidase

A high-resolution 3D crystal structure of the 2009 pandemic H1N1 influenza neuraminidase (PDB: 3TI5) complexed with Zanamivir was retrieved from Protein Data Bank (Berman et al., 2002). The criteria for choosing PDB structure included (a) minimum resolution and (b) conformation of docked ligand being the same as in the crystallized structure after the redocking procedure. The structure of influenza neuraminidase existed as a dimer, as such, only chain A of the enzyme was selected for further analysis to reduce computational time and cost. To prepare the selected X-ray structure of the enzyme, co-crystallized water molecules, small molecules, nonpolar hydrogens, lone pairs and nonstandard residues were deleted, followed by an addition of hydrogens and Gasteiger charges. Preparations of was in accordance with in-house preparation protocols performed on the UCSF Chimera software (Pettersen et al., 2004).

Virtual Screening

As a widely employed computational approach, virtual screening allows for the screening of potential inhibitors against a given target from a large database of chemical compounds (Lionta et al., 2014). This also allows for the pruning of down of the number of potential inhibitors to a number that can easily be managed and further analyzed. Structure-Based Virtual Screening (SBVS) as employed in this study was utilized to screen DrugBank database (Wishart et al., 2008) in the quest of finding novel and potential hit compounds. Retrieved 2D structure of Zanamivir was uploaded onto the Drug Bank chemical structure screening tool (Wishart et al., 2008). Results were filtered to include only compounds that are similar in structure to Zanamivir while meeting the requirements of the Lipinski Rule of 5 (Ro5) (Lipinski et al., 2001).

Ligand Acquisition and Preparation

Compounds yielded from the virtual screening were extracted in 2D form DrugBank. The extracted compounds were individually opened in Avogadro (Hanwell et al., 2012) and energy minimization performed using the MMF94 force field. The minimized 3D structures were thereafter individually exported to UCSF Chimera for further preparation prior to molecular docking. Hydrogen atoms were removed and Geister partial charges were allocated to the compounds.

Molecular Docking

To ensure that compounds would bind effectively with the active site, the retrieved compounds were individually docked into the active site of influenza neuraminidase using Autodock Vina software (Trott and Olson, 2010). In SBVS, molecular docking predicts the binding conformation of inhibitors to the binding site of the selected target. Description of the binding mode plays an essential role in the rational design of drugs as well as to expounding on the essential biochemical processes. The Lamarckian Genetic Algorithm was used to generate the docked complexes, as it is widely accepted as a suitable docking method (Morris et al., 1998). To enclose the active site of neuraminidase, a gridbox size of 38 X 24 X 32 Å was utilized. The docked complexes of each compound were visualized using ViewDock plugin integrated in UCSF Chimera. Docked results were ranked according to their binding affinities.

In silico Pharmacokinetic Analysis

To further assess suitability of retrieved compounds, it was necessary to determine their pharmacokinetic properties. In this regard, the SMILES of the 3 compounds were extracted from ZINC Database and uploaded into the online tool Admet-SAR 2 (Cheng et al., 2012). The tool was utilized to determine if the retrieved compounds possessed drug-like qualities, by assessing their compatibility with the RO5, its lipophilicity and its membrane permeability. These properties, together with the binding affinities of each compound, were used to create the final ranking of the retrieved compounds.

Determination of Interactions between Ligand and Active Site

Ligand interaction plots of the obtained hit compounds and Zanamivir were generated using MAESTRO software (Maestro 10.2 User Manual, 2015) to assess the types of interactions between the ligand and active site for both compounds. Using these plots, the interactions and number of hydrogen bonds present between the ligand and the active site for both the reference compound and the hit were compared. This comparison helped to draw conclusions as to whether the hits would theoretically be a better inhibitors than Zanamivir.

Results and Discussion

The aim of the current study was to elucidate alternative inhibitory compounds against the H1N1 Influenza neuraminidase. In silico virtual screening of the DrugBank database of compounds was performed by establishing a pipeline of the Lipinski’s rule of five and
pharmacokinetic properties to assess drug likeness. Compounds that showed a strong binding affinity for Influenza neuraminidase were selected for further investigations. Further, the ADMET profile was analyzed to filter top hits to identify suitable virtual hit candidate compounds. The current in silico study was undertaken to identify efficient anti-influenza virus inhibitors. The virtual hits identified in this study can be used as an alternative targeting-agent for H1N1 influenza virus upon further experimental investigations. Prior to screening the DrugBank database for potential neuraminidase inhibitors, the binding site residue interaction profiles of Zanamivir within the binding pocket of neuraminidase was analyzed. As such, the crystal structure of the H1N1 influenza neuraminidase-inhibitor Zanamivir inhibitor (PDB ID: 3TI5) was obtained from PDB an uploaded on the Maestro software to reveal the residue interacting profile with the co-crystalized Zanamivir. It was found that residues Glu228, Trp179, Arg152, Glu277, Arg378 and Glu119 formed significant interactions with the Zanamivir as shown in Fig. 3.

Structure-based Virtual Screening

To identify the new anti-influenza virus inhibitors that targeted the neuraminidase, 2D structure of Zanamivir was uploaded on the screening interface of the DrugBank online tool. Screening involved the applications of a series of filters (Ro5, drug likeness, structural similarity), which led to the discovery three compounds: DB03321, DB11888 and DB12791 as shown in Fig. 4. Molecular docking of all three compounds to the neuraminidase binding pocket was performed to estimate the binding affinities. Binding affinities of the hits were compared with that of Zanamivir, the known neuraminidase inhibitor as shown Table 1. Docking all 3 potential inhibitors into the active site of influenza neuraminidase (Fig. 5) revealed docking scores of -6.3 kcal/mol, -0.69 kcal/mol and -7.9 kcal/mol for DB12791, DB11888 and DB03321 respectively. Compound DB03321 exhibited the highest binding affinity of -7.9 kcal/mol relative to the other two potential inhibitors. All three hits were further analyzed for their pharmacokinetic properties after exhibiting favorable binding affinities from the docking.

Comparative In silico Pharmacokinetic Analysis of Hits

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) of compounds serve as molecular descriptors that determine the pharmacokinetic properties of these compounds. The in silico prediction of ADMET properties allows the identification of the prospects of hits to be used as human therapeutic agent (Moroy et al., 2012). The admetSAR online server was utilized for calculating the ADMET properties of the three hits obtained from the screening. It was observed that all three hits possessed favorable pharmacokinetic properties as shown in Table 1.

![Fig. 3: Chemical structure of zanamivir](image1)

![Fig. 4: Influenza neuraminidase-zanamivir interaction plot. Purple arrows indicate hydrogen bonds formed with residues at the active site](image2)
Fig. 5: Chemical structures of 3 potential inhibitors of neuraminidase, yielded from DrugBank, based on the structural properties of zanamivir

Table 1: The DrugBank codes, 2D structures, docking score and Lipinski’s rule of five for the top docked compounds

<table>
<thead>
<tr>
<th>DrugBank Code</th>
<th>∆G (kcal/mol)</th>
<th>ALogP</th>
<th>Molecular Weight (Da)</th>
<th>Donors</th>
<th>Acceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zanamivir</td>
<td>-8.1</td>
<td>-6.80</td>
<td>348.31</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>DB03321</td>
<td>-7.9</td>
<td>-5.15</td>
<td>290.27</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>DB11888</td>
<td>-6.9</td>
<td>-3.16</td>
<td>472.54</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>DB12791</td>
<td>-6.3</td>
<td>-6.08</td>
<td>346.34</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

DB03321 possessed the highest binding affinity at the active site, as well as favorable pharmacokinetic properties, such as a low AlogP value and the lowest molecular weight, implying that the compound was most likely to be soluble in biological fluids and permeate membranes, in order to reach the site of action (Du et al., 2016). Therefore, it was singled out from the three hits for further investigation. To buttress the impressive pharmacokinetic profile and the estimated binding affinities obtained from the docking procedure for DB03321, the intermolecular interactions exhibited by DB03321 was also explored with particular interest on the number of hydrogen bond formed. Hydrogen bonds are among the most important intermolecular forces and stronger than van der Waals interactions due to their high associated energies (Steiner, 2002). Therefore, a greater number of hydrogen bonds observed between molecules portrays the strength of the binding.

A comparison of the ligand-interaction plot of DB03321 at the binding site relative to the interactions engaged in by Zanamivir as shown in Fig. 4 revealed that DB03321 formed more hydrogen bonds with residues within the binding site. Inhibitor interactions plots of the DB03321are shown in Fig. 6. DB03321 engaged in 10 hydrogen bond interactions with binding sites residues relative to Zanamivir, which engaged in only 7 hydrogen bonds suggesting DB03321 bound more strongly to neuraminidase. Hence, it can be predicted that since DB03321 bound strongly to binding site in neuraminidase due to the higher number of hydrogen bonds, it could possibly exhibit better inhibitory activity as a promising potential inhibitor upon further experimental exploration.

Overall, Zanamivir appears superior to the hit, based on its higher binding affinity and favourable pharmacokinetic profile. However, the reports Zanamivir resistant strains calls for the discovery of new compounds (Hurt et al., 2009). Conversely, DB03321, the hit compound, has comparable favourable binding affinity and pharmacokinetic properties and no known reports of resistance and therefore could be a starting point for further investigation towards the continuous search for novel influenza neuraminidase inhibitors.

The current study is an effort to identify anti-influenza compounds that target neuraminidase that may be considered for drug development to influenza virus infections. These virtual hits followed the LRo5 and drug likeness and did not violate any of the rules indicating they may not lead to any issues associated with bioavailability, hence could further be experimentally investigated for their prospects. Overall, all potential inhibitors discovered showed good human intestinal absorption and blood-brain barrier accessibility considering the favorable lipophilicity that they demonstrated. The hits also assumed favorable poses with then binding pocket of influenza neuraminidase in a similar pose as Zanamivir as shown in Fig. 7 after molecular docking.
Fig. 6: Influenza neuraminidase-Zanamivir interaction plot highlighting prominent hydrogen bond interactions

Fig. 7: Superimposition of three hits; DB03321 (yellow), DB11888 (magenta) and DB12791 (green) docked into the binding site of the enzyme target, influenza neuraminidase with Zanamivir (red)

The binding affinity of the hits as obtained from the molecular docking coupled with their respective half-life measures the efficiency of the inhibitor–enzyme complex. The rate of dissociation of a given inhibitor within the pocket is faster if it possesses weaker intermolecular interactions and equally slower when the interactions are stronger (Boyer et al., 2007). As such, the higher hydrogen bond formed in the case of DB03321 relative to Zanamivir, which exhibited fewer interactions, suggest a possible longer half-life of DB0332 and stability within the pocket, hence will take longer to dissociate, hence a possible enhanced inhibitory potency. In the influenza neuraminidase-DB003321 complex, the residues with the binding pocket that were engaged in these strong hydrogen bond interactions include; Glu278, Glu277, Arg293, Arg118, Glu119, Glu228, Asp151, Arg152 and Trp179.

Conclusion

The influenza virus, commonly known as flu, is spread through air droplets, when an infected person coughs, sneezes or spits. The cumulative resistance to current anti-influenza treatments poses a serious threat for world health. To this end, a computational study was conducted and was aimed at finding novel potential inhibitors of influenza neuraminidase, based on the structural properties of the reference drug Zanamivir, with potentially increased binding affinity for the active site and reduced resistance. Three potential inhibitors obtained from the DrugBank database screening are reported on in this study. Amongst the three, DB03321 was predicted to be the best based on its binding energy at the active site and possession of drug like properties.
It was therefore selected for further analysis, which revealed it was more stable at the active site than the reference drug due to the greater number of hydrogen bonds it formed with vital residues in the binding pocket. The results of this study could serve as a starting point in the search for novel neuraminidase inhibitors. Advanced computational studies such as molecular dynamics simulations could serve as validation for this in silico research. Further in vitro and in vivo investigation of this compound could lead the way in the development of improved neuraminidase inhibitors towards the treatment of Influenza virus.

Conflict of Interest

The authors declare no conflict of interest.

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Author’s Contributions

Sumaiyah Motala, Nasiphi Magaqa, Sandile Ndlovu, Nobuhle Dlamini, Shabile Mkhide and Thabiso Siyaya contributed by literature reviews, data analysis, interpretation, manuscript writing and preparation of graphics while Clement Agoni and Fisayo A. Olotu contributed to manuscript editing and as Supervisors.

Ethics

No animal or human models were used in the research.

References
