Computational Insights into the Drug Repurposing and Synergism of FDA-approved Influenza Drugs Binding with SARS-CoV-2 Protease against COVID-19

Shazia Parveen1,*, Rua B. Alnoman1, Abrar A. Bayazeed2, Alaa M. Alqahtani3

1Faculty of Science, Chemistry Department, Taibah University, Yanbu Branch, 46423, Yanbu, Saudi Arabia
2Chemistry Department, Faculty of Applied Science, Umm Al-Qura University, 21955, Makkah, Saudi Arabia
3Pharmaceutical Chemistry Department, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia

*Corresponding author: shazia021@gmail.com

Received July 20, 2020; Revised July 31, 2020; Accepted August 10, 2020

Abstract The concept of drug repurposing is extensively used currently to identify already approved/under investigation/discarded potential drugs for various other diseases, owing to the fact that many drugs could have multiple protein targets and many diseases share overlapping molecular pathways. The geographical spread of COVID-19 infections originating from Wuhan, China, has provided an opportunity to study the natural history of the recently emerged virus. The source of the SARS-CoV-2 is yet not known, even though the initial cases have been connected with the Huanan South China Seafood Market. In this study, the bioactivity of FDA-approved influenza drugs (Baloxavir, Oseltamivir, Peramivir and Zanamivir) were evaluated as inhibitors for COVID-19 using computational modeling approaches. The nominated drugs were docked on SARS-CoV-2 main protease (PDB ID: 6LU7) and also with SARS HCoV (PDB ID: 6NUR) for comparison, to evaluate the binding affinity of these drugs. ADMET and DFT analyses were also further carried out to analyze the potential of these influenza drugs as an effective inhibitor against COVID-19. The DFT calculations were performed to estimate the thermal parameters, dipole moment of the investigated drugs; additionally, chemical reactivity descriptors were investigated. The results of molecular docking with respect to binding energies in Kcal/mol suggested that binding affinity of influenza drugs with SARS-CoV-2 was in the order Zanamivir > Baloxavir > Oseltamivir > Peramivir. The findings of this study can facilitate rational drug design targeting the SARS-Cov-2 main protease.

Keywords: COVID-19, influenza drugs, 3CLpro, molecular docking, DFT


1. Introduction

Globally, as of 4 July 2020, there have been 10,902,637 confirmed cases of COVID-19, including 522,446 deaths, reported to WHO (https://covid19.who.int/). In late December, 2019, a report was published on ProMED-mail reporting a bunch of patients with pneumonia like symptoms of unknown etiology in Wuhan city, Hubei Province, China (https://promedmail.org/). On February 12, 2020, this disease caused by the novel coronavirus was officially named as 2019 novel coronavirus (2019-nCoV)/Coronavirus Disease 2019 (COVID-19) by WHO.

The virus responsible for COVID-19 has been recognized as a novel enveloped RNA betacoronavirus2; currently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), having phylogenetich similarity to SARS HCoV [1]. Human coronaviruses are positive-sense, long (30,000 bp) single-stranded RNA viruses belonging to the family Coronaviridae, capable of causing various disease with enteric, respiratory, hepatic and neurological symptoms [2]. Two groups of proteins characterize coronaviruses- i) structural proteins such as Spike, nucleocapsid, matrix and envelope, in addition to the nonstructural proteins, such as proteases and ii) RdRp (RNA-dependent RNA polymerase) [3]. The Spike protein that is present on the virus outer surface in a homo-trimeric state; is a fundamental recognition factor for the attachment and entry of virus into the host cells [4]. The results of this novel coronavirus genome sequencing, in combination with other reports revealed it’s 75 to 80% identical nature to the SARS HCoV but having closer relation to several bat coronaviruses [5]. It can be transmitted in the same cells that are useful for growing SARS HCoV but notably, SARS-Cov-2 grows better in primary human airway epithelial cells than in standard tissue-culture cells, unlike SARS HCoV [6]. Presently, the number of established contagions has been increasing on a daily-basis, but there is no definite treatment for the...
pneumonia and other symptoms caused by SARS-CoV-2, although some potential drugs are under investigation worldwide. The severity of COVID-19, needs an urgency to develop a vaccine within the record breaking time of approximately 12-18 months.

The process for a new drug development, preclinical research and approval is expensive and time-taking, can take up to a decade. This lengthy discovery process unlocks the doors for drug repurposing as an alternative approach for cutting down the time required to develop a drug [7]. Even though this approach is not new, it has gained substantial impetus in the last decade: about one-third of the approvals in recent years correspond to drug repurposing, generating around 25% of the annual revenue for the pharmaceutical industry [8].

Since, COVID-19 is a respiratory condition causing fever, fatigue, dry cough, muscle aches, shortness of breath, pneumonia [9,10]. In severe cases, it could lead to ARDS (Acute respiratory distress syndrome) characterized by severe lung inflammation in which the fluid builds up in the vicinity and within the lungs causing septic shock due to dramatic fall in blood pressure and bodily organs are starved for oxygen. Incubation period of this corona virus is approximately 1 to 14 days [11].

Keeping this in view, the present study focused on drug repurposing approach of FDA-approved influenza drugs- Baloxavir [12], Oseltamivir [13], Peramivir [14] and Zanamivir [15] (Figure 1) for COVID-19, the main proteases in coronaviruses [16,17,18], especially PDB ID: 6LU7, as potential target proteins for COVID-19 treatment.

SARS-CoV-2 is a positive sense RNA virus that requires RNA templated RNA synthesis [19]. Hence, we explored the effect of synergism of investigated drugs against the SARS-CoV-2 3CL main protease. Since, the SARS HCoV is the closest strain to the newly emerged SARS-CoV-2, it is used as docking target for comparison [20,21,22]. The results are promising and suggest possible inhibition for the currently available therapeutics against the newly emerged coronavirus.

Figure 1. Chemical structures of investigated FDA-approved influenza drugs
2. Materials and Methods

2.1. *In silico* ADME and Drug-likeness

Physically significant descriptors and pharmaceutically relevant properties of all the test compounds like molecular weight, mlogP, H-bond donors, and H-bond acceptors according to the Lipinski’s rule of five were analyzed. The ADMET properties of the influenza drugs were also analyzed using SwissADME (http://www.swissadme.ch/index.php) [23]. Computational analyses to predict the core pharmacokinetics parameters such as blood-brain barrier (BBB) permeability, gastrointestinal (GI) absorption, P-glycoprotein-mediated efflux (Pgp) were also performed.

2.2. Molecular Docking

The molecular docking studies were performed by using AutoDock Vina [24]. The crystal structures of SARS CoV-2 (PDB ID: 6LU7) [25] and SARS HCoV (PDB ID: 6NUR) [26] were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) in PDB format and were prepared by AutoDock Tools [27]. A grid box (40 Å × 40 Å × 40 Å) centered at (-25.986, 12.590, 59.154) Å and (149.982, 147.534, 157.026) Å, for the SARS-CoV-2 and SARS HCoV, respectively, was used in the docking experiments. Visualization of the docked poses has been done by CHIMERA (www.cgl.ucsf.edu/chimera) and Discovery Studio visualizer.

2.3. DFT Calculations

Density functional theory (DFT) has been proved to be a powerful tool for the study of electronic and thermodynamic parameters. The quantum computational studies of influenza drugs were performed in gas phase on GAMESS [28,29]. Considering the complexity of the theoretical model used for this study, all the structural optimizations were carried out at the DFT using a basis set of B3LYP/3-21G method. The electronic properties of the drugs such as $E_{\text{HOMO}}$, $E_{\text{LUMO}}$, HOMO-LUMO energy gap, global hardness, electronegativity, electronic chemical potential, electrophilicity and chemical softness, natural charges and dipole moment were calculated [30,31,32].

3. Results and Discussions

3.1. *In silico* ADME and Drug-likeness

The major parameters for pharmacokinetics are absorption, distribution, metabolism and excretion [33]. The *in silico* predicted ADME properties and their values of influenza drugs are shown in Table 1. The results of *in silico* properties suggests that there are no significant violations of Lipinski’s rule of five [34], since all calculated physicochemical descriptors and pharmacokinetic properties are within the expected thresholds. According to this model, the drugs showed satisfactory oral bioavailability in combination with lipophilicity, MW, polarity, solubility, saturation, flexibility in acceptable range as shown in radar plots (Figure 2) [35].

![Figure 2](image-url) Bioavailability radar plot of drug (a) Baloxavir, (b) Oseltamivir, (c) Peramivir and (d) Zanamivir. POLAR (polarity), LIPO (lipophilicity), INSOLU (solubility), FLEX (flexibility), and INSATU (saturation)
Table 1. Selected calculated physicochemical and pharmacokinetic properties of investigated FDA-approved influenza drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>mLogP</th>
<th>TPSA (Å²)</th>
<th>MW (g/mol)</th>
<th>nON</th>
<th>nOHNH</th>
<th>n violations</th>
<th>Nrotb</th>
<th>GI absorption</th>
<th>BBB permeant</th>
<th>Pgp substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baloxavir</td>
<td>2.64</td>
<td>124.84</td>
<td>571.55</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>High</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>0.63</td>
<td>90.65</td>
<td>312.40</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>High</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Peramivir</td>
<td>0.01</td>
<td>148.53</td>
<td>328.41</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>Low</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Zanamivir</td>
<td>-3.18</td>
<td>200.72</td>
<td>332.31</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>Low</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

mLogP = lipophilicity; TPSA = Total Polar Surface Area, Å²; MW = Molecular Weight; nON = number of hydrogen bond acceptors; nOHNH = number of hydrogen bond donors; n violations = number of violated drug-likeness rules; nrotb = number of rotating bonds, BBB = blood-brain barrier; GI = gastrointestinal; Pgp = p-glycoprotein.

Drug-likeness was determined by number of free rotatable bonds and Lipinski’s rule. Computational analyses to predict the core pharmacokinetics parameters such as gastrointestinal absorption, P-glycoprotein-mediated efflux was also performed and results are displayed in Table 1.

3.2. Molecular Docking

Molecular docking provides a paramount source for analyzing the binding properties of compounds based on the computer-aided analysis [21,22,36]. Molecular docking of FDA-approved influenza drugs, Baloxavir, Oseltamivir, Peramivir and Zanamivir, with the SARS-CoV-2 main protease were performed individually. Also SARS HCoV was used as docking targets for comparison. The binding of ligand in the active site of a target is indicative of the probability that the ligand may possibly be capable of steering functional alteration of the target molecules [37,38]. Drug-target interactions were also decoded in terms of interacting amino acid residues, hydrogen bonding, docking energy analysis and comparisons of active site amino acid residues and probable binding sites.

The Figure 3 displays the interacting residues between influenza drugs and respective targets. From the Figure 3, we can say that the four FDA-approved influenza drugs (Baloxavir, Oseltamivir, Peramivir and Zanamivir) (orange sticks) were found to bind to SARS-CoV-2 with binding energies -7.2, -6.3, -5.8 and -9.2 Kcal/mol respectively. These drugs were able to bind to the new coronavirus strain (SARS-CoV-2) firmly and therefore could possible contradict the polymerase function. Also they were found to bind with SARS HCoV with binding energies, ΔG = -6.2, -6.8, -5.6 and -7.0 Kcal/mol respectively. From the binding energies, we could conclude that all four drugs, interacted with SARS-CoV-2 more firmly as compared to older strain (SARS HCoV). The binding energies gave us an insight into the affinity of the drug to the target. The binding affinity of influenza drugs with SARS-CoV-2 was in the order Zanamivir > Baloxavir > Oseltamivir > Peramivir.

The docking analysis of influenza drugs within the active binding sites of targets are shown in Table 2 and Figure 4.

![Figure 3. Molecular docking interaction of (a) Baloxavir, (b) Oseltamivir, (c) Peramivir and (d) Zanamivir displaying amino acids residues with SARS-CoV-2 (COVID-19) (PDB ID: 6LU7) and SARS HCoV (PDB ID: 6NUR)](image-url)
Table 2. Molecular docking analysis of investigated FDA-approved influenza drugs within the binding sites of SARS-CoV-2 (PDB ID: 6LU7) and SARS HCoV (PDB ID: 6NUR) and their binding energies in Kcal/mol

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interacting amino acid residues (Binding energies in Kcal/mol)</th>
<th>SARS-CoV-2 (PDB ID: 6LU7)</th>
<th>SARS HCoV (PDB ID: 6NUR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baloxavir (Xofluza)</td>
<td>[Image of Baloxavir interactions]</td>
<td>[Image of Baloxavir interactions]</td>
<td>[Image of Baloxavir interactions]</td>
</tr>
<tr>
<td>Oseltamivir (Tamiflu)</td>
<td>[Image of Oseltamivir interactions]</td>
<td>[Image of Oseltamivir interactions]</td>
<td>[Image of Oseltamivir interactions]</td>
</tr>
<tr>
<td>Peramivir (Rapivab)</td>
<td>[Image of Peramivir interactions]</td>
<td>[Image of Peramivir interactions]</td>
<td>[Image of Peramivir interactions]</td>
</tr>
</tbody>
</table>
Hydrogen bonding were also evaluated involved in hydrogen bonding of influenza drugs for interaction of influenza drugs with targets. Table 3 summarizes the amino acid residues within the binding sites of SARS-CoV-2 and SARS HCoV.

Table 3. Binding energies in Kcal/mol and amino acid residues involved in hydrogen bonding of investigated FDA-approved influenza drugs within the binding sites of SARS-CoV-2 and SARS HCoV

<table>
<thead>
<tr>
<th>Drug</th>
<th>SARS-CoV-2 (PDB ID: 6LU7)</th>
<th>SARS HCoV (PDB ID: 6NUR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding energy (Kcal/mol)</td>
<td>Receptor residues involved in Hydrogen bonding</td>
</tr>
<tr>
<td>Baloxavir</td>
<td>-7.2</td>
<td>Gln110, Lys102, Ser158, Thr111, Thr292</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>-6.3</td>
<td>Asp153, Gln110</td>
</tr>
<tr>
<td>Peramivir</td>
<td>-5.8</td>
<td>Gln110, Thr111</td>
</tr>
<tr>
<td>Zanamivir</td>
<td>-9.2</td>
<td>Asn151, Gln110, Thr111, Thr292</td>
</tr>
</tbody>
</table>

Ala-alanine; Arg-arginine; Asn-asparagine; Asp-aspartic acid; Cys-cysteine; Gln-glutamine; Lys-lysine; Ser-serine; Thr-threonine; Trp-tryptophan; Tyr-tyrosine; Val-valine.
Total binding strength is an result of many types of bonds including ionic, hydrophobic interactions and Vander Waals forces, though hydrogen bonds being major contributor [39,40]. Hydrogen bonding also depends on the composition and 3D alignment of contacting amino acid residues at the prominent and active binding sites [41]. The results of docking analysis revealed that all the four drugs were able to form hydrogen bonding interactions with amino acid residues in the active sites. As we can see from Table 2 and Table 3, Baloxavir was capable of hydrogen bonding with Gln110, Lys102, Ser158, Thr111 and Thr292. While Oseltamivir could form hydrogen bonds with Asp153, Gln110. Peramivir was able to form hydrogen bonding with only two amino acid residues Gln110 and Thr111. While the most potent drug, Zanamivir was capable of forming hydrogen bonds with Asn151, Gln110, Thr111 and Thr292. From these findings, we could conclude that the variations due to the presence of contacting amino acid residues common to the active binding sites, with little relationship to presence or absence of hydrogen bonds. This proposed that the presence of the hydrogen bonds was independent with the commonness of contacting amino acid residues to active binding sites and strength of docking.

### 3.3. DFT Calculations

Density functional theory is a computer-based approach that has been gaining huge popularity in the field of in silico pharmaceutical analysis. It was performed to analyze the electronic and reactivity characteristics of the influenza drugs. The analysis of the electronic characteristics of the ligands plays a central role in understanding their pharmacological properties. Figure 5, displays the DFT optimized structures of influenza drugs. Frontier molecular orbitals (FMOs) are the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO).

The HOMO is the highest energy orbital occupied with electrons, so it is an electron donor, while, LUMO is the lowest energy orbital that has a space to accept electrons, so it is an electron acceptor. These orbitals control the mode of the interaction of the drugs with other molecules such as the interactions between these drugs and their receptors. Further, these drugs were analyzed on the basis of HOMO/LUMO energy gap. As the gap energy increases, it leads to decrease reactivity and vice versa [42]. B3LYP functional method was applied for DFT calculations, while for calculating band energy gap, $\Delta E$ expression of $E_{\text{LUMO}} - E_{\text{HOMO}}$ was used [43]. The results are summarized in Table 4.

![Figure 5. DFT-optimized geometry of (a) Baloxavir, (b) Oseltamivir, (c) Peramivir and (d) Zanamivir](image-url)
Table 4. The theoretical calculated conceptual DFT descriptors of investigated FDA-approved influenza drugs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baloxavir</th>
<th>Oseltamivir</th>
<th>Peramivir</th>
<th>Zanamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{HOMO}}$ (a.u.)</td>
<td>-0.298</td>
<td>-0.371</td>
<td>-0.333</td>
<td>-0.319</td>
</tr>
<tr>
<td>$E_{\text{LUMO}}$ (a.u.)</td>
<td>0.091</td>
<td>0.093</td>
<td>0.155</td>
<td>0.096</td>
</tr>
<tr>
<td>$\Delta E_{\text{LUMO-HOMO}}$</td>
<td>0.389</td>
<td>0.464</td>
<td>0.488</td>
<td>0.415</td>
</tr>
<tr>
<td>Electronegativity ($\chi$)</td>
<td>0.1035</td>
<td>0.1390</td>
<td>0.0890</td>
<td>0.1150</td>
</tr>
<tr>
<td>Chemical hardness ($\eta$)</td>
<td>0.1945</td>
<td>0.2320</td>
<td>0.2440</td>
<td>0.2075</td>
</tr>
<tr>
<td>Chemical softness ($\sigma$)</td>
<td>5.117</td>
<td>4.310</td>
<td>4.098</td>
<td>4.819</td>
</tr>
<tr>
<td>Chemical potential ($\mu$)</td>
<td>-0.1035</td>
<td>-0.139</td>
<td>-0.089</td>
<td>-0.115</td>
</tr>
<tr>
<td>Electrophilicity index ($\omega$)</td>
<td>-0.0275</td>
<td>-0.0415</td>
<td>-0.0161</td>
<td>-0.0318</td>
</tr>
<tr>
<td>Nucleophilic index ($\varepsilon$)</td>
<td>-0.0201</td>
<td>-0.0322</td>
<td>-0.0217</td>
<td>-0.0238</td>
</tr>
</tbody>
</table>

Figure 6. HOMO-LUMO energy of optimized structure of (a) Baloxavir, (b) Oseltamivir, (c) Peramivir and (d) Zanamivir

Table 5. Thermal parameters (Hartree/Particle) (KJ/mol) and Dipole moment (Debye) of investigated FDA-approved influenza drugs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baloxavir</th>
<th>Oseltamivir</th>
<th>Peramivir</th>
<th>Zanamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZPVE</td>
<td>1386.105</td>
<td>1219.210</td>
<td>1238.562</td>
<td>971.890</td>
</tr>
<tr>
<td>$E_{\text{tot}}$</td>
<td>1462.559</td>
<td>1274.597</td>
<td>1294.769</td>
<td>1024.711</td>
</tr>
<tr>
<td>$H$</td>
<td>1465.038</td>
<td>1277.076</td>
<td>1297.248</td>
<td>1027.190</td>
</tr>
<tr>
<td>$G$</td>
<td>1235.710</td>
<td>1093.185</td>
<td>1111.655</td>
<td>848.821</td>
</tr>
<tr>
<td>$\mu$</td>
<td>5.510841</td>
<td>4.315624</td>
<td>4.767919</td>
<td>6.108949</td>
</tr>
</tbody>
</table>

Figure 6 shows the HOMO-LUMO energies of the investigated influenza drugs. The thermodynamic properties were also calculated and summarized in Table 5. The dipole moment in a molecule is another important electronic property. Whenever the molecule has larger dipole moment, the intermolecular interactions are very strong [44]. The dipole moments of the investigated drugs was calculated to be in the order Zanamivir (6.108949 Debye) > Baloxavir (5.510841 Debye) > Peramivir (4.767919 Debye) > Oseltamivir (4.315624 Debye), with an insignificant difference between Peramivir and Oseltamivir. These findings were in corroboration with the findings of molecular docking studies, suggesting that potent drug Zanamivir exhibited strong intermolecular interactions.

Besides the traditional reactivity descriptors (HOMO and LUMO), certain other chemical reactivity descriptors such as chemical hardness ($\eta$), chemical softness ($\sigma$), electrophilicity index ($\omega$), nucleophilic index ($\varepsilon$), electronegativity ($\chi$) and chemical potential ($\mu$) were also calculated and are summarized in Table 4 [30,31,32]. All these factors could share together with different extent to significantly impact the degree of the binding affinity of these drugs with the active drug targets.

4. Conclusion

To battle the fatal corona virus infection, numerous studies are ongoing using influenza drug therapies. Four
FDA-approved influenza drugs, Baloxavir, Oseltamivir, Peramivir and Zanamivir have been investigated as inhibitors for COVID-19 by molecular docking and DFT calculations. Molecular docking was performed to analyze the binding interaction of the investigated influenza drugs with the COVID-19 target SARS-CoV-19 (PDB ID: 6LU7), compared with SARS HCov (PDB ID: 6NUR). The results showed several hydrogen bonding interactions,
with the active amino acid residues with the binding energies of -7.2, -6.3, -5.8 and -9.2 Kcal/mol for Baloxavir, Oseltamivir, Peramivir and Zanamivir, respectively. The selected drugs exhibited good binding interaction with the targets. DFT analysis of the investigated drugs also showed satisfactory outcomes. Thus, these influenza drugs, viz., Baloxavir, Oseltamivir, Peramivir and Zanamivir, can be analyzed through the experiment in the future clinical trials of drugs against SARS-CoV-2.

Abbreviations

ADME: absorption, distribution, metabolism and excretion
ARDS: Acute respiratory distress syndrome
Bp: base pair
Cov: Coronavirus
COVID-19: Coronavirus Disease 2019
DFT: density functional theory
FDA: Food and drug administration
FMO: frontier molecular orbital
HOMO: highest occupied molecular orbital
LUMO: lowest unoccupied molecular orbital
MW: molecular weight
PDB: protein data bank
RdRp: RNA-dependent RNA polymerase
RNA: ribonucleic acid
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
SARS HCov: severe acute respiratory syndrome
WHO: World health organization

Acknowledgments

SP and RBA are thankful towards Taibah University.

Conflict of Interest

The authors declare no conflicts of interest.

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