umsurabaya
UNIVERSITAS MUHAMMADIYAH SURABAYA
Camellia
Clinical, Pharmaceutical, Analitical and Pharmacy Community Journal
http://journal.um-surabaya.ac.id/index.php/CAM

In silico Study on Physicochemical, Pharmacokinetic and Toxicity Profiles of Available Antiviral Drugs and The Drug-Target Interaction with Protease of SARS-CoV-2

Studi *In Silico* Profil Fisikokimia, Farmakokinetik dan Toksisitas Obat Antivirus yang Beredar dan Interaksi Obat-Target dengan Protease SARS-CoV-2

Nuzul Wahyuning Diyah[1]*, Aileen Syifa Ghifari[1], Shabrina Wahyu Hidayati[2], Juni Ekowati[1]

[1]Faculty of Pharmacy Universitas Airlangga, Surabaya, Indonesia
[2]Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia
**Corresponding author* : nuzul-w-d@ff.unair.ac.id

ARTICLE INFO

Received:
7 October 2022

Revised:
14 November 2022

Accepted:
25 December 2022

Published Online:
31 December 2022

ABSTRACT

Introduction: In emergency conditions such as the COVID-19 pandemic, many drugs treating SARS-CoV-2 are currently being developed through the concept of repurposing the existing drugs. This study aims to evaluate the physicochemical, pharmacokinetic and toxicity (ADMET) profiles of 15 drugs that have been used clinically for other viral diseases, and inspect their activity as antivirals against SARS-CoV-2. **Methods:** The physicochemical properties were obtained by using the ChemDraw and the ADMET were predicted using pkCSM on line tools. The selected drugs based on Lipinski's Rules were docked into main protease of SARS-CoV-2 (PDB. 6LU7) using Molegro program. **Results:** Six drugs complied with Lipinski's Rules and showed good ADMET profile except for their hepatotoxicity, but favipiravir and oseltamivir were predicted to be non-hepatotoxic. Oseltamivir also showed high binding affinity with free energy score below -100 kcal/mol. **Conclusions:** Oseltamivir is a potential antivirus for COVID-19 based on physicochemical, ADMET profile, and in silico activity against protease of SARS-CoV-2.

Keywords: ADMET, antiviral drugs, in silico, physicochemical, SARS-CoV-2

ABSTRAK

Pendahuluan: Dalam kondisi darurat seperti pandemi COVID-19, banyak obat yang dapat digunakan untuk mengobati SARS-CoV-2 yang saat ini sedang dikembangkan melalui konsep *repurposing* obat yang sudah ada. Penelitian ini bertujuan untuk mengevaluasi profil fisikokimia, farmakokinetik, dan toksisitas (ADMET) dari 15 obat yang telah digunakan secara klinis untuk penyakit virus lainnya, dan memeriksa aktivitasnya sebagai antivirus terhadap SARS-CoV-2. **Metode:** Karakteristik fisikokimia diperoleh dengan menggunakan ChemDraw dan ADMET diprediksi menggunakan perangkat pkCSM on line. Obat-obatan yang dipilih berdasarkan Aturan Lipinski dimasukkan ke dalam protease utama SARS-CoV-2 (PDB. 6LU7) menggunakan program Molegro**. Hasil:** Enam obat sesuai dengan Aturan Lipinski dan menunjukkan profil ADMET yang baik kecuali untuk hepatotoksisitasnya, namun favipiravir dan oseltamivir diprediksi tidak hepatotoksik. Oseltamivir juga menunjukkan

afinitas pengikatan yang tinggi dengan nilai energi bebas di bawah -100 kkal/mol. Kesimpulan: Oseltamivir adalah antivirus potensial untuk COVID-19 berdasarkan fisikokimia, profil ADMET, dan aktivitas in silico terhadap protease SARS-CoV-2.
Kata kunci: ADMET, obat antivirus, in silico, fisikokimia, SARS-CoV-2

INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic had a major impact on the health emergency and the global economic problem. More than one hundred and a quarter of deaths and nearly 2 million confirmed cases have been reported globally, making the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 virus an urgent public health concern (Vankadari, 2020). The sudden emergency and continuous spread of this new coronavirus has resulted in an urgent need for the development of methods for the rapid diagnosis of the SARS-CoV-2 virus and the treatment of the infectious disease COVID-19. Doctors have controlled the COVID-19 virus infection using few available drugs and the results obtained subsequently showed variations in the level of control. Scientists around the world are striving to find drugs for combating this disease (Chhikara et al., 2020).

So far, research have revealed more than 30 agents including modern medicines, natural products, and traditional medicines that have potential efficacy against COVID-19 (Dong et al, 2020). Several antiviral drugs are being evaluated to repurpose as possible treatments for COVID-19 (Li and De Clercq, 2019). Some of these agents have been rapidly tested in clinical studies and demonstrated preliminary efficacy against COVID-19. The use of available antiviral drugs in the clinical treatment of COVID-19 is currently still based on their potential as antiviral agents against other viral diseases or based on the result of in vitro experiment, drug screening in silico and an enzyme activity test.

The repurposing antiviral drugs are mostly anti-retroviral agents (ARV) used to treat HIV including atazanavir (AZV), darunavir (DRV), lopinavir (LPV), raltegravir (RGV), ritonavir (RTV), saquinavir (SQV), tipranavir (TPV) (Dong, 2020) which known as protease inhibitors, and tenofovir (TNV) which is different class of ARV (Vankadari, 2020). Some of the drugs under clinical trial are anti-influenza which work through different mechanism of action, including baloxavir (BLV) (Lou et al, 2020), favipiravir (FPV) (Cai et al., 2020) oseltamivir (OSV) (Wu et al, 2020), and umifenovir (UMV) (Wang et al., 2020). The other antivirals proposed to be clinically tested for treat COVID-19 are nelfinavir (NLV) (Musarrat et al, 2020), dolutegravir (DGV) (Indu et al., 2020), and letermovir (LMV) (Pathak et al, 2020).

ARV agents inhibit the replication of retroviruses, especially HIV, through several mechanisms of action, including as integrase inhibitors, nucleoside reverse inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors (Safrin, 2009). The use of ARVs are usually combined with several antiretroviral drugs to be more effective than monotherapy. Lopinavir/ritonavir are protease inhibitors that have in vitro antiviral activity against SARS and in clinical studies (Chu et al, 2004). Darunavir (DRV) is a second-generation of HIV-1 protease inhibitor that could inhibiting HIV replication. The study to evaluate the antiviral activity and safety of darunavir/cobicistat (DRV/c) in treating COVID-19 concluded five days of DRV/c did not increase the proportion of negative conversion compared with standard of care alone (Chen et al, 2020).

Favipiravir which was approved for treatment of influenza is a new type of RNA-dependent RNA polymerase (RdRp) inhibitor. In addition to its anti-influenza virus activity, favipiravir is capable of blocking the replication of another RNA viruses (Delang et al, 2018). Therefore, favipiravir may have potential antiviral action on SARS-CoV-2, which is an RNA virus. This drug is currently undergoing clinic trials in treating COVID-19 in China (Jean et al, 2020). The study carried out at a hospital in Istanbul with the data of 179 inpatients to have a diagnosis of COVID-19 receiving therapy combined with favipiravir demonstrated a proper safety profile. However, its side effects such as teratogenicity have not yet been adequately studied. It may be safe and tolerable in short-term use, but more evidence is needed to assess the longer-term effects of treatment (Yilmaz et al., 2020).

Oseltamivir is approved oral drug for the treatment and prevention of influenza types A and B. Its active metabolite, oseltamivir carboxylate, inhibit neuraminidase which results in viral aggregation at the surface of the cell and decreases virus spread within the respiratory tract (Acosta, 2018). The in vitro activity of oseltamivir against SARS-CoV-2 has not been documented. Coronaviruses do not have neuraminidase (Fehr and Perlman, 2015) and after replicating inside the host cell, the virus needs the help of viral protein E and the

exocytosis to escape (Dou et al, 2018). However, oseltamivir was given empirically during the initial outbreak of COVID-19 in China before the discovery of the causative virus SARS-CoV-2 (Wang et al., 2019).

Umifenovir (arbidol), another antiviral drug that can be used as anti-influenza, inhibited crucial stages of the SARS-CoV-2 replication cycle in vitro in a concentration of 10-30 μM (Dong et al., 2020). The predominant mode of action of umifenovir is inhibition of membrane fusion between virus particles and plasma membranes, and between virus particles and the membranes of endosomes. Alternatively, umifenovir may also be immunomodulatory and capable of interferon induction and/or macrophage activation. Due to such broad-spectrum antiviral activities, umifenovir represents a promising candidate for treatment of viral infections in humans (Haviernik et al, 2018).

Limited clinical studies of favipiravir showed some efficacy in COVID-19 treatment with tolerable adverse effects (Yilmaz et al, 2020). Oseltamivir has no documented activity against SARS-CoV-2 even though it was given empirically in the early outbreak of COVID-19 in China (Wang et al., 2019), while lopinavir/ritonavir showed limited efficacy in COVID-19 (Chen et al, 2020). There has been no sufficient evidence to recommend any specific treatment for COVID-19. Several old drugs that have been used for other indications, or new drugs that are still under trials, are being studied in various parts of the world. The efficacy and safety data of drugs used for COVID-19 are limited and most of the research are still ongoing (Wu, 2020). Repurposing old and relatively safe drugs became an advantageous option to obtain the urgently needed effective treatment.

There are three strategies for repurposing antivirus (Mercorelli et al., 2018); the first is antivirals acting on the same target at different viruses (Reznik et al., 2018). Second, drugs work on the same target but for new indications, such as the anticancer drug imatinib which works by inhibiting Abelson kinase (Abl kinase), it was found to have in vitro inhibitory activity of the SARS-CoV and MERS-CoV coronaviruses in Vero cells which also involved Abl kinase (Sisk et al., 2018). Third, a new target approach for new indications, for example the antiparasitic drug ivermectin which works by binding the glutamate-gated chloride channel in muscle and nerve cells of invertebrate organisms, causing paralysis (Rizzo, 2020). Subsequent studies reported that ivermectin can provide in vitro antiviral activity against SARS-CoV-2 in Vero-hSLAM cells (Formiga et al., 2020).

The fifteen antiviral drugs mentioned above are compounds which work with different mechanisms of action repurposed to treat the same virus, SARS-CoV-2, so that more than one approach of repurposing was applied. The target selected for in silico study of these available antiviral drugs were main protease (M-pro or 3CL-pro) of SARS-CoV-2 which is the 3C protease homologue of the picorna virus, a cysteine protease enzyme that plays an important role in the maturation process of functional polypeptide in the replication of the SARS-CoV-2 (Cui et al, 2020). Inhibition of M-pro will inhibit the replication of the SARS-CoV-2 so that the number of viruses could be suppressed. This viral protease has no homologues with human proteases (Joshi et al., 2020) so that it can be a specific target for antiviral drugs which only act on viruses and has minimal toxic effects on human cells.

In drug development study, biological activity is influenced by physicochemical properties of drugs that undergo structural changes when undergoing pharmacokinetic processes of absorption, distribution, mechanism, excretion (ADME) before arriving at the site of action and interacting with receptors/target. Because these drugs were developed to target the same virus (SARS-CoV-2), it is necessary to evaluate their physicochemical properties, pharmacokinetic, and toxicity profiles before exploring their activity as antiviral against SARS-CoV-2.

Considering the current public health crisis, this study was conducted to evaluate the physicochemical properties, pharmacokinetic and toxicity (ADMET) profiles of fifteen antiviral agents (AZV, BLV, DRV, DGV, FPV, LMV, LPV, NLV, OSV, RGV, RTV, SQV, TPV, TNV, UMV) for supporting the development of antiviral drugs in treatment of COVID19 by concept of repurposing. To predict the potential activity as an antiviral against SARS-CoV-2, the molecular docking on main protease was performed for compounds that fulfilled physicochemical and ADMET criteria.

METHODS

Materials

Fifteen available antiviral drugs were used as test compounds, the names were listed in Table I along with physicochemical property. This in silico study used Asus laptop supported by processor intel CORES i5 CORE i5 8th Gen RAM 4 GB and equipped with computer programs including ChemDraw Pro 16.0, Chem 3D 16.0, Molegro Virtual Docker (MVD) ver 5.5, and Discovery Studio Visualizer (DSV). Two-

dimensional structure (2D) of the fifteen antiviral drugs as test compounds and their SMILES format were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Physicochemical properties

The 2D structure of the test compounds were processed by ChemDraw Pro 16.0 to obtain their physicochemical properties including molecular weight (MW), partition coefficient (log P), water solubility (log S) as counterpart of log P, molar refractivity (MR). The 2D structures were converted to three dimension (3D) and subjected to energy minimization by MMFF94 calculation using Chem3D 16.0 program prior to analyze the number of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). MW, Log P, HBD, and HBA are physicochemical properties required for prediction of oral bioavailability of drugs according to Lipinski's Rules of Five (Lipinski et al, 2012).

Pharmakokinetics and Toxicity Prediction

The pharmacokinetic properties (absorption, distribution, metabolism, excretion) and toxicity profile of the test compounds were determined by pkCSM on line tools (http://biosig.unimelb.edu.au/pkcsm/prediction) using molecules in SMILES format.

Molecular Docking

The compounds studied for in silico molecular docking were those whose physicochemical properties have complied with Lipinski's Rules of Five.

Ligand Preparation.

The 3D structure of each compounds that have been processed by Chem3D 16.0 to obtain optimized geometry were stored as mol2 file. The molecules in this format were already in the minimum energy conformation dan available as test ligands to be imported into the workspace of MVD program for docking simulation.

Protein preparation.

The target protein in the pdb format was obtained from the Protein Data Bank (PDB) (www.rcsb.org) before subsequent preparation with the MVD 6.0. The target protein was main protease (M-pro) of the SARS-CoV-2 (PDB.6LU7) which contains original ligand N-[(5-methylisoxazol–3–yl)carbonyl]alanyl–L–valylN~1~((1R,2Z)–4-(benzyloxy)–4–oxo–1-{[(3R)–2–oxopyrrolidin–3yl]methyl}but–2-enyl)-L-leucinamide (code: PRD) that is an inhibitor of M-pro. The protein molecule was imported into workspace of MVD then prepared to detect the ligand's binding site (cavity). The procedure yielded two files, including the prepared protein (protein.pdb) and extracted original ligand (ori_ligand.mol2).

Docking procedure.

The validation of docking procedure was done by re-docking the the prepared original ligand (ori_ligand.mol2) into the binding site of M-

Table I Physicochemical properties of test compounds

No	Compound	MW	Log P	MR	Log S	HBA	HBD
1	Atazanavir	704.39	5.921	19.685	-7.265	8	5
2	Baloxavir	483.11	2.583	12.344	-5.339	8	1
3	Darunavir	547.24	2.880	14.428	-4.756	7	3
4	Dolutegravir	419.38	0.407	10.167	-4.428	6	2
5	Favipiravir	157.10	-1.518	3.363	-0.395	4	2
6	Letermovir	572.20	7.058	14.312	-8.279	11	1
7	Lopinavir	628.81	6.094	18.233	-7.136	5	4
8	Nelfinavir	567.79	5.842	16.411	-7.369	6	4
9	Oseltamivir	312.41	2.131	8.586	-2.007	4	2
10	Raltegravir	444.42	-0.645	10.981	-4.340	10	2
11	Ritonavir	720.95	4.937	20.118	-7.034	9	4
12	Saquinavir	670.86	4.727	19.128	-6.935	**7**	5
13	Tenofovir	287.08	-1.563	66.467	-0.676	7	3
14	Tipranavir	602.67	7.764	15.495	-8.802	6	2
15	Umifenovir	477.42	5.496	5.242	-6.044	4	1

Notes: MW in g/mol and MR in cc/mol

pro (protein.pdb). The method was applied when the root mean square value (RMSD) of re-docked ligand< 2.0 Å. The docking results were MolDock scores (MDS) which served as free energy ligand-protein complex. The same procedure was applied to each test ligands by introducing them into the binding site of original ligand. The docking scores of test ligands (Table 3) were selected from the best scoring pose of ligand interacting with the amino acid residue of the protein. The ligand-protein complexes with the lowest docking scores were used for further visual inspection using DSV program.

RESULTS AND DISCUSSION

Physicochemical properties

The physicochemical properties of fifteen antiviral drugs are listed in Table 1 and evaluated by Lipinski's Rules of Five (Lipinski et al, 2012). Data in the table showed that baloxavir, dolutegravir, favipiravir, oseltamivir, raltegravir,

Table II Pharmacokinetic and toxicity profiles of test compounds

IIA. Absorption and distribution

No	Compound	Absorption		Distribution		
		Caco-2 Permeability[1)]	Intestinal absorption	VDss[2)] (human)	BBB permeability[3)]	CNS permeability[4)]
1	Atazanavir	1.420	83.5 %	0.193	-1.290	-2.921
2	Baloxavir	0.717	52.9 %	-0.296	-0.667	-3.073
3	Darunavir	0.191	59.4 %	0.617	-1.360	-3.476
4	Dolutegravir	1.044	86.7 %	-0.442	-1.172	-3.486
5	Favipiravir	1.164	95.0 %	-0.362	-0.472	-3.126
6	Letermovir	0.618	67.9 %	-0.003	-0.614	-1.967
7	Lopinavir	1.357	89.4 %	-0.044	-0.946	-3.026
8	Nelfinavir	0.285	64.0 %	0.709	-0.624	-2.189
9	Oseltamivir	1.001	75.3 %	-0.154	-0.312	-3.424
10	Raltegravir	0.517	87.5 %	-0.723	-1.485	-3.317
11	Ritonavir	0.897	73.1 %	-0.122	-1.916	-3.486
12	Saquinavir	0.888	71.4 %	0.494	-0.952	-3.693
13	Tenofovir	0.491	65.5 %	-0.162	-1.721	-3.683
14	Tipranavir	0.658	59.2 %	0.226	-1.401	-2.925
15	Umifenovir	-0.332	57.6 %	0.874	-0.089	-2.193

IIB. Metabolism, excretion, and toxicity

No	Compound	Metabolism		Excretion	Toxicity		
		CYP3A4	CYP2D6	Clearance[5)]	Rat LD_{50} [6)]	Rat LD_{50} Chronic[7)]	Hepato-toxicity
1	Atazanavir	No	No	0.596	1626	2.703	Yes
2	Baloxavir	S, I	No	0.382	1349	1.036	Yes
3	Darunavir	S, I	No	0.749	1631	2.775	Yes
4	Dolutegravir	S, I	No	0.107	1081	1.393	Yes
5	Favipiravir	S, I	No	0.148	420	2.023	No
6	Letermovir	No	No	0.926	1289	1.490	Yes
7	Lopinavir	I	No	0.778	2027	5.949	Yes
8	Nelfinavir	S, I	Yes	0.653	1115	3.911	Yes
9	Oseltamivir	No	No	-0.047	679	1.091	No
10	Raltegravir	S	No	-0.097	1105	1.562	Yes
11	Ritonavir	S	No	0.494	1642	2.231	Yes
12	Saquinavir	No	No	0.728	1277	2.597	Yes
13	Tenofovir	S, I	No	0.549	864	2.358	Yes
14	Tipranavir	S, I	No	0.270	2007	2.326	Yes
15	Umifenovir	No	I	0.709	1059	0.737	Yes

Notes: 1) log Papp in 10^{-6} cm/s, 2) (Log L/kg), 3) log BB, 4) log PS, 5) total clearance (log ml/min/kg), 6) g/kg body weight, 7) g/kg bw/day, S= substrate, I= inhibitor

tenofovir, and umifenovir met the molecular weight criteria. There were 9 drugs that met the requirement of Log P, including the six previously drugs except umifenovir, and the next 3 drugs were darunavir, ritonavir, saquinavir. All compounds fulfilled the HBA criteria, and almost all suitable for HBD requirement except letermovir.

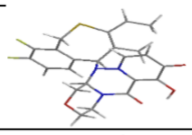
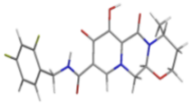
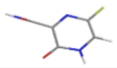
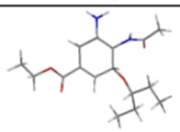
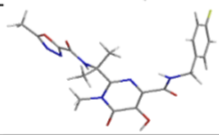
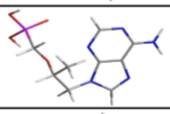
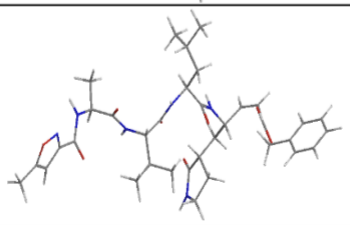
The results indicated six drugs that is baloxavir, favipiravir, dolutegravir, oseltamivir, raltegravir and tenofovir complied with Lipinski's Rules of Five, that is MW< 500, log P< 5, HBD< 5, HBA< 10. Based on log P data, most of the compounds were lipophilic, supported by data of solubility in water (log S) which showed that only 3 compounds can be soluble in water (log S> -4), that is favipiravir, oseltamivir, and tenofovir (Quimque et al., 2020). Data MR indicated that favipiravir, oseltamivir, and tenofovir have smaller molecular size compare with the other compounds (MR< 10 cc/mol).

Pharmacokinetic and Toxicity Profile

The pharmacokinetic and toxicity (ADMET) profile of test compound obtained by using pkCSM online tool are listed in Table II. Based on data of absorption and distribution (Table IIA), among the 6 compounds that met Lipinski's Rule, favipiravir, dolutegravir, and oseltamivir were those that could
penetrate cell membranes (Caco-2 permeability> 0.9) but all compounds could be absorbed through the intestine (intestinal absorption > 30%). Distribution data showed darunavir, nelfinavir, and umifenovir were compounds that have high distribution volume (Vd > 0.45), all compounds were not easily distributed through blood brain barrier (BBB) because all of the Log BB values were negative, and all compounds also not easily distributed to the central nervous system (CNS permeability < -2). The only compound that was predicted to penetrates the CNS was letermovir.

According to the metabolism profile in the Table IIB, oseltamivir which complies with Lipinski's Rules was one of the compounds that were neither a substrate nor inhibitor for the selected CYP isozyme, besides atazanavir, letermovir, and saquinavir. In related with toxicity, oral LD50 for acute toxicity of all test compounds were very high (> 15 g) so that the drugs were classified as level 6 compound which is practically not toxic or relatively harmless (BPOM RI, 2004) because these drugs have been approved for clinical used as antiviral against influenza virus, as anti-retroviral against HIV, and an antiviral against cytomegalovirus, i.e. letermovir (Safrin, 2009). However, all of them are hepatotoxic except oseltamivir and favipiravir.

Table III Molecular structure and docking score of selected compounds against main protease SARS-CoV-2

Code	3D Molecular Structure	Docking score (kcal/mol)
BLV		-29.80
DGV		-78.51
FPV		-65.19
OSV		-100.61
RGV		-110.67
TNV		-104.71
PRN*		-169.23

Notes: *PRN is the original ligand in 6LU7, serves as reference ligand

Docking Result

Molecular docking of test compounds as ligand in binding site cavity-1 (volume= 117.76 Å) of main protease of SRAS-CoV-2 (PDB. 6LU7) yield docking scores as representation of free energy ligand-protein complexes (kcal/mol) are displayed in Table III.

Based on docking score (MolDock score) in the Table III, raltegravir, tenofovir, and oseltamivir showed high binding affinity for the M-pro of SARS-CoV-2, respectively. Interaction of the two selected ligand with amino acids in binding site (cavity-1) displayed in Figure 1. Raltegravir (RGV) was the best docked ligand while oseltamivir (OSV) was that showed best ADMET profiles among the three top score ligand. However, the affinity of the ligand to the protease is still lower than that of PRD, the original ligand which is an inhibitor for M-pro and a co-crystal in the M-pro structure.

Among the corona viral targets that have been studied in the past, the main protease (M-

pro, 3CL-pro, nsp5) received major attention. The main protease M-pro exclusively cleaves polypeptide sequences after a glutamine residue, so that the main protease is an ideal drug target because no human host-cell proteases are known with this substrate specificity. The SARS-CoV-2 M-pro proteolytically cleaves the overlapping polyproteins (pp1a and pp1ab) to functional proteins, which is a critical step during viral replication. Replication-essential enzymes such as RdRp or nsp13 cannot fully function without prior proteolytic release thereby positioning M-pro as a key enzyme in the viral replication cycle. Consequently, its inhibition can block the production of infectious viral particles and thus alleviate disease symptoms (Ullrich and Nitsche, 2020). Therefore, compounds that can inhibit M-pro will be able to block viral activity. The best docking pose of six test ligands in the 3D structure of M-pro can be seen in Figure 1. The position of all docked ligands in the binding site was same as original ligand PRD although the molecular structure of the compounds (Table 3) were quite varies.

In general, six compounds interacted with the same amino acid in the M-pro cavity-1 (Figure 1). Further inspection in 2D interaction showed hydrogen bond (H-bond) built by ligands and amino acids, including: baloxavir with Cys145 (2 H-bonds), dolutegravir with Gly143, Ser144, Cys 145, Oseltamivir with Ser144, favipiravir with Cys145 (2 H-bonds) and His164, raltegravir with Asn142, Ser144, Leu141, and tenofovir with Gly 143, Ser144, Cys 145.

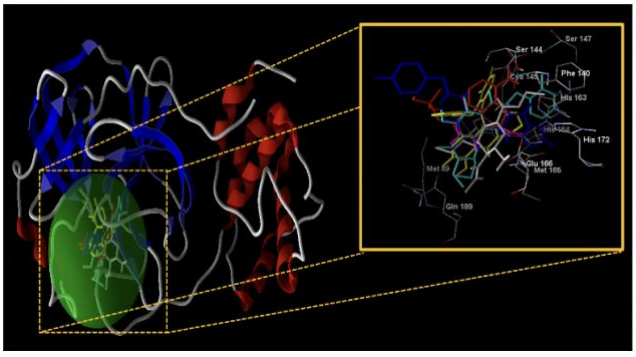


Figure 1. Binding interaction of 6 selected compounds (BLV, DGV, FPV, OSV, RGV, TNV) with amino acids at the binding site of M-pro SARS-CoV-2, shown in 3D image of the protein secondary structure. Picture in the yellow box is the best docking position of test ligands in stick format compared with original ligand PRD (navy blue stick)

The binding interactions of high scoring ligands, i.e. OSV and RGV are displayed in Figures 2. In Figure 2, top scored ligand (RGV) showed more hydrogen bond than the third ranked ligand (OSV) but the OSV displayed more van der Waals interaction with amino acids in the binding site of the protease. Oseltamivir also showed electrostatic interaction with Glu166, beside a hydrophobic interaction with Phe140, while raltegravir only exhibited hydrophobic interaction with Met165.

Although the original target of oseltamivir in influenza virus is neuraminidase, it turns out that oseltamivir has a higher affinity for M-pro than baloxavir and favipiravir which were also antiviral drugs for influenza medication.

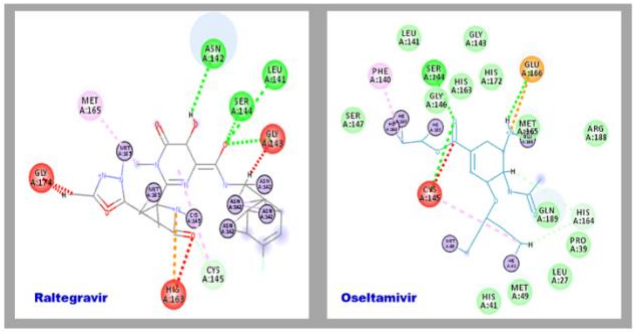


Figure 2. The 2D interaction of selected ligand (OSV, RGV, TNV) with amino acids of M-pro SARS-CoV-2 (PDB. 6LU7). The green dashed line represents the hydrogen bond, pink line is hydrophobic interaction, amino acids in pale green denoted van der Waals interaction with functional group of the compound, orange line is electrostatic interaction, and the red-line is unfavorable bump.

In this study, raltegravir, tenofovir, and oseltamivir were antiviral drugs that complied with Lipinski's Rules of Five and exhibit higher binding affinity for the M-pro of the SARS-CoV-2 among the five selected compounds. Oseltamivir was also showed better ADMET profiles compare with them.

CONCLUSIONS

This study proposed oseltamivir as a potential agent to be developed for antiviral drug in treatment of COVID19 based on its physicochemical characteristics, ADMET profile, and in silico activity againts main protease SARS-CoV-2. Oseltamivir is one of the drugs currently in clinical use for COVID-19 patients.

Generally, there are no finally verified antivirals specific to COVID-19 at present. The efficacy and safety of these candidate drugs in the treatment of COVID-19 need to be confirmed in further preclinical and clinical trials.

ACKNOWLEDGMENT

Thankfullness to Prof. Dr. apt. Siswandono, M.S from Faculty of Pharmacy who facilitated the use of the Molegro Virtual Docker program.

REFERENCES

Acosta, P.E., (2018). Antiviral agents (nonretroviral). In L.L. Brunton, R. Hilal-Dandan, B.J. Knollmann (Eds). *Goodman & Gilman's the pharmacological basis of therapeutics* 13th ed. (pp. 1105–1116). McGraw-Hill Education, New York.

BPOM Republic of Indonesia (National Agency of Drug and Food Control). (2014). Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia No. 7 tahun 2014 tentang Pedoman Uji Toksisitas Nonklinik Secara In Vivo.

Cai, Q., Yang, M., Liu, D., Chen J., Shu, D., Xia, J., Liao, X., Gu, Y., Cai, Q., Yang, Y., Shen, C., Li, X., Peng, L., Huang, D., Zhang, J., Zhang, S., Wang, F., et al. (2020). Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. *Engineering*, 6(10): 1192-1198.

Chen, J., Xia, L., Liu, L., Xu, Q., Ling, Y., Huang, D., Huang, W., Song, S., Xu, S., Shen, Y., and Lu, H. (2020). Antiviral Activity and Safety of Darunavir/Cobicistat for the Treatment of COVID-19. Open Forum Infectious Diseases: 1-5.

Chhikara, B. S., Rathi, B., Singh, J., & Poonam, F. N. U. (2020). Corona virus SARS-CoV-2 disease COVID-19: Infection, prevention and clinical advances of the prospective chemical drug therapeutics. *Chemical Biology Letters*, 7(1), 63–72.

Chu, C.M., Cheng, V.C.C., Hung, I.F.N., Wong, M.M.L., Chan, K.H., Chan, K.S., Kao, R.Y.T., Poon, L.L.M., Wong, C.L.P., Guan, Y., Peiris, J.S.M., Yuen, K.Y. (2004). Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. *Thorax*, 59:252-256.

Cui, W., Yang, K., Haitao Yang, H. (2020). Recent Progress in the Drug Development Targeting SARS-CoV-2 Main Protease as Treatment for COVID-19. *Frontiers in Molecular Biosciences* 7:1–10.

Delang, L., Abdelnabi, R., Neyts, J., Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. (2018). Antiviral Resesearch, 153:85-94.

Dong, L., Hu, S., Gao, J. (2020). Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discoveries & Therapeutics*, 14(1):58-60.

Dou, D., Revol, R., Östbye, H., Wang, H., Daniels, R. (2018). Influenza A virus cell entry, replication, virion assembly and movement. *Front Immunology*, 20(9):1581.

Fehr, A.R., Perlman S. (2015) Coronaviruses: an overview of their replication and pathogenesis. Methods Molecular Biology,1282:1–23.

Formiga, F.R., L., De Souza, J., Paiva, L. (2020). Ivermectin: an award-winning drug with expected antiviral activity against COVID-19. *Journal of Controlled Release*, 10(329): 758–761.

Haviernik, J., Štefánik, M., Fojtíková, M., Kali, S., Rudolf, I., Hubálek, Z., Eyer, L., Ruzek, D., Tordo, N. (2018). Arbidol (Umifenovir): A Broad-Spectrum Antiviral Drug That Inhibits Medically Important Arthropod-Borne Flaviviruses. *Viruses*, 10(184): 1-8.

Indu,P., Rameshkumar, M.R., Arunagirinathana, N., Al-Dhabi, N.A., Mariadhas Valan Arasud, M.V., Ignacimuthu, S. (2020). Raltegravir, Indinavir, Tipranavir, Dolutegravir, and Etravirine against main protease and RNA-dependent RNA polymerase of SARS-CoV-2: A molecular docking and drug repurposing approach. *Journal of Infection and Public Health*, 13(12):1856–1861.

Joshi, R.S., Jagdale, S.S., Bansode, S.B., Shankar, S.S., Tellis, M.B., Pandya, V.K., Chugh, A., Giri, A.P., Kulkarni, M.J.. (2021). Discovery of Potential Multi-Target-Directed Ligands by Targeting Host-Specific SARS-CoV-2 Structurally Conserved Main Protease. *Journal of Biomolecular Structure and Dynamics,* 39(9):3099-3114

Li, G., De Clercq, E. (2020). Therapeutic options for the 2019 novel coronavirus (2019-nCoV) *Nature reviews Drug Discovery*,19:149-150.

Lipinski, C. A., Lombardo, F., Dominy, B.W., Feeney, P.J. (2012). Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews* 64(SUPPL.):4–17.

Lou, Y., Liu, L., Yao, H., Hu, X., Su, J., Xu, K., Luo, R., He, L., Lu, X., Zhao, Q., Liang, T., Qiu, Y., First, T., (2020). Clinical Outcomes and Plasma Concentrations of Baloxavir Marboxil and Favipiravir in COVID-19 Patients: An Exploratory Randomized, Controlled Trial. *European Journal of Pharmaceutical Sciences*, 157:105631.

Mercorelli, B., Palù, G., Arianna Loregian, A. (2018). Drug Repurposing for Viral Infectious Diseases: How Far Are We? *Trends in Microbiology*, 26(10): 865–876.

Musarrat, F., Chouljenko, V., Dahal, A., Nabi, R., Chouljenko, T., Jois, S.D., Kousoulas, K.G. (2020). The anti-HIV drug nelfinavir mesylate (Viracept) is a potent inhibitor of cell fusion caused by the SARSCoV-2 spike (S) glycoprotein warranting further evaluation as an antiviral against COVID-19 infections. *Journal of Medical Virology*: 1-9.

Pathak, Y., Mishra, A., Choudhir, G., Kumar, A., Tripathi, V. (2021). Rifampicin and Letermovir as potential repurposed drug candidate for COVID-19 treatment: insights from an in-silico study. *Pharmacological Reports*, 1-13.

Quimque, M. T. J., Notarte, K. I. R., Fernandez, R. A. T., Mendoza, M. A. O., Liman, R. A. D., Lim, J. A. K., Wei, D. Q. (2020). Virtual Screening-Driven Drug Discovery of SARS-CoV2 Enzyme Inhibitors Targeting Viral Attachment, Replication, Post-Translational Modification and Host Immunity Evasion Infection Mechanisms. *Journal of Biomolecular Structure and Dynamics*, 39(12): 4316–4333.

Reznik, S.E., Tiwari, A.K., Ashby, C.R. (2018). Sofosbuvir: A Potential Treatment for Ebola. *Frontiers in Pharmacology,* 9: 2016–2019.

Safrin, S. (2009). Antiviral Agents. In B.G. Katzung, S. Masters, A.J. Trevor (Eds), *Basic and Clinical Pharmacology* 11th ed. (pp. 853-857). McGraw Hill Medical, New York.

Sisk, J.M., Frieman, M.B., Machamer, C.E. (2018). Coronavirus S Protein-Induced Fusion Is Blocked Prior to Hemifusion by Abl Kinase Inhibitors. *Journal of General Virology,* 99(5): 619–630.

Rizzo, E. (2020). Ivermectin, Antiviral Properties and COVID-19: A Possible New Mechanism of Action. *Naunyn-Schmiedeberg's Archives of Pharmacology,* 393(7):1153–1156.

Ullrich, S., and Nitsche, C. (2020). The SARS-CoV-2 main protease as drug target. *Bioorganic & Medicinal Chemistry Letters*, 30:127377.

Vankadari,N. (2020). Arbidol: A potential antiviral drug for the treatment of SARS-CoV-2 by blocking trimerization of the spike glycoprotein. *International Journal of Antimicrobial Agents*, 56:105998.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2019). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*, 323(11):1061–1069.

Wang, Z, Chen X, Lu Y, Chen, F., Zhang, W.(2020). Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. *Bioscience Trends,* 14:64–68.

Wu, R., Wang, L., Kuo, H-C.D., Shannar, A., Peter, R., Chou, P.J., Li, S., Hudlikar, R., et al. (2020). An Update on Current Therapeutic Drugs Treating COVID-19. *Current Pharmacology Reports*, 11: 1–15.

Wu, X., Cai, Y., Huang, X., Yu, X., Zhao, L., Wang F., et.al. Co-infection with SARS-CoV-2 and influenza A virus in patient with pneumonia, China. (2020) *Emerging Infectious Disease*, 26(6):1324–1326.

Yilmaz, H., Guner, E., Altuntas, M. (2020). Results of Favipiravir Combined Treatment in Intensive Care Patients With Covid-19. *Research Square*, 1-11.