



IDENTIFICATION OF NEW POTENTIAL SARS-COV-2 PROTEINS INHIBITORS THROUGH VIRTUAL SCREENING AND MOLECULAR DOCKING SIMULATIONS

Navjot K. Virk, Monika*

Dept. of Biotechnology, Mata Gujri College, Fatehgarh Sahib, Sirhind, Punjab, India

*Corresponding author: monika@matagujricollege.org, navjotkaurvirk12@gmail.com

Received: 10-01-2022; Accepted: 18-03-2022; Published: 30-04-2022

© Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License <https://doi.org/10.55218/JASR.202213308>

ABSTRACT

The pathogen Coronavirus is a member of the Orthocoronavirinae subfamily of the Coronaviridae family and the Nidovirales order. Four structural proteins, in addition to viral RNA and Nsp1-16, are employed to speed up virus replication within host cells. Antiviral drugs that target the S protein have recently been discovered, although the technique of targeting the S protein has some limitations. The objective of the study was to test the potential inhibitors for the use of novel agents against CoV-2 infection. In this, we conducted the virtual screening of various lead compounds from three different databases i.e. SWEETLEAD, ZINC, and Selleckchem Antiviral compound library against COVID-19 proteins Nsp 15 and Nsp 7 and 8 complex which act as receptors using two software like Molegro Virtual Docker and PyRx. For this, a phytochemical library was constructed followed by ligand and target preparation. The molecular docking and Virtual screening as well as ADMET characteristics were also analyzed. Our results revealed twenty high-affinity lead compounds from 4,200 molecules out of which Danoprevir, Lanatoside C, and Digoxin/ZINC242548690 can act as powerful inhibitors of Nsp 15 and Lanatoside C, Ledipasvir, and BMS-790052 for Nsp 7 and 8 complex targets respectively.

Keywords: COVID-19, Molinspiration, Molegro Virtual Docker, Non-Structural Proteins, Selleckchem Antiviral Compound Library, SWEETLEAD, Virtual Screening.

1. INTRODUCTION

The World Health Organization (WHO) was alerted by the China Health Authority in December 2019 about a couple of instances of pneumonia of unknown origin in Wuhan, Hubei, central China. The swab test from the patients' throats revealed a novel coronavirus, which was initially curtailed 2019-nCoV by WHO [1]. Coronavirus is associated with a high mortality rate [2]. Fever, weakness, and cough were the most common symptoms of COVID-19, which were similar to those seen in SARS-CoV and MER patients [3]. Coronavirinae members are split into four genera: α - and β -coronaviruses, which can infect humans, while Gamma and Delta-coronaviruses are viruses that have only been detected in animals. Gamma-coronaviruses infect whales and birds, while Delta-coronaviruses infect pigs and birds [4]. COVID-19 is a contagious respiratory infection that spreads via globules, respiratory secretions, and spitting [5, 6]. The treatment of patients infected with SARS-CoV-2 has received considerable attention [7, 8].

Prevention of viral RNA production, delaying replication of the virus, obstructing virus binding to receptors of the host cells, and impeding the virus's process of self-assembly are all anti-coronaviral techniques [9]. The ACE2 receptor is abundant throughout the respiratory tract and in salivary epithelial cells and is shown to be a SARS-CoV early target [10]. Spike (S), membrane (M), envelope (E), and nucleocapsid (N) are four significant coronaviral structural proteins, encoded by the viral genome's 3' [11], along with 16 non-structural proteins (Nsp) [12]. Their involvement in coronavirus replication makes these NSP proteins good candidates for anti-coronavirus medication development. COVID-19 therapy may be possible if one or more Nsp proteins are targeted [13]. During the COVID-19 outbreaks, a number of prospective therapeutics for SARS-CoV-2 have been proposed, with Remdesivir, hydroxychloroquine, lopinavir, ritonavir, etc. being the most promising. In October 2020, the Food and Drug Administration (FDA) approved Remdesivir as the first and only

medicine to treat COVID-19 [14]. By targeting the SARS-CoV-2 RdRp enzyme, Remdesivir has been found to shorten the recovery period for patients hospitalized with COVID-19 [15]. It has been proven that lopinavir and ritonavir are effective in treating patients with SARS, which is caused by the viral major protease (Mpro) [16]. Protein-ligand binding energy prediction was recently applied to uncover potential COVID-19 medications using molecular docking and virtual screening approaches [17, 18]. Many lead compounds generated from natural sources and existing medications have been investigated against a number of proteins, including Nsps and structural proteins, to see if they can target SARS-CoV-2 [19-22].

SWEETLEAD, ZINC, and Selleckchem Antiviral compound libraries were screened for lead compounds that may have inhibitory activity against the SARS-CoV-2 Nsp 15 and Nsp 7 and 8 complex. In addition to Remdesivir, other pharmacological options with suppressive action and lower binding energies with the SARS-CoV-2 proteins were investigated. Four thousand and two hundred molecules extracted from these databases were docked and virtually screened.

2. EXPERIMENTAL

2.1. Preparation of proteins

The Nsp 15 and Nsp 7-8 complex was chosen as the primary targets for this work and their 3D structures were obtained from the Protein Data Bank (PDB). The proteins that were chosen were prepped for docking. Protein preparation was carried out using the BIOVIA Discovery studio's hierarchy and chemistry visualization tools, as well as the Molegro virtual docker's prepare protein tool.

2.2. Ligand library generation

Three databases were used to retrieve ligands, including SWEETLEAD, ZINC, and the Selleckchem library. We picked 4,200 medicines from these databases for Virtual Screening. The ligand preparation was done in Biovia Discovery Studio and Molegro virtual docker.

2.3. Virtual screening and molecular docking

Molegro Virtual Docker was utilized for docking. Remdesivir was considered as the reference drug. The grid box was set for Nsp 15 and Nsp 7-8 complexes. Then, molecular structures of 4,200 medicines from the three databases were screened for promising candidates against Nsp 15 and Nsp 7-8 complex of SARS-CoV-2 virus. The best pose was chosen when docking was

completed. Hydrogen bond interactions and Backbone visualization was done.

2.4. Selection of lowest energy molecules

Twenty molecules were chosen for each target based on the lowest energy value via Microsoft Excel.

2.5. Cross validation of selected compounds

This step was performed using PyRx software. Initially, the standard molecule Remdesivir was independently validated in PyRx for Nsp 15 and Nsp 7 and 8 complex, and then twenty chosen molecules were selected for docking with each target.

2.6. ADMET analysis

For ADMET testing, three compounds were chosen. These compounds were chosen based on their lowest binding energy. Molinspiration was utilized to investigate the ADMET properties of a few molecules.

3. RESULTS AND DISCUSSION

A total of 4,200 compounds were used in this investigation, which were gathered from several sources. Using docking technologies like Molegro Virtual Docker, these compounds were virtually screened against two coronavirus targets.

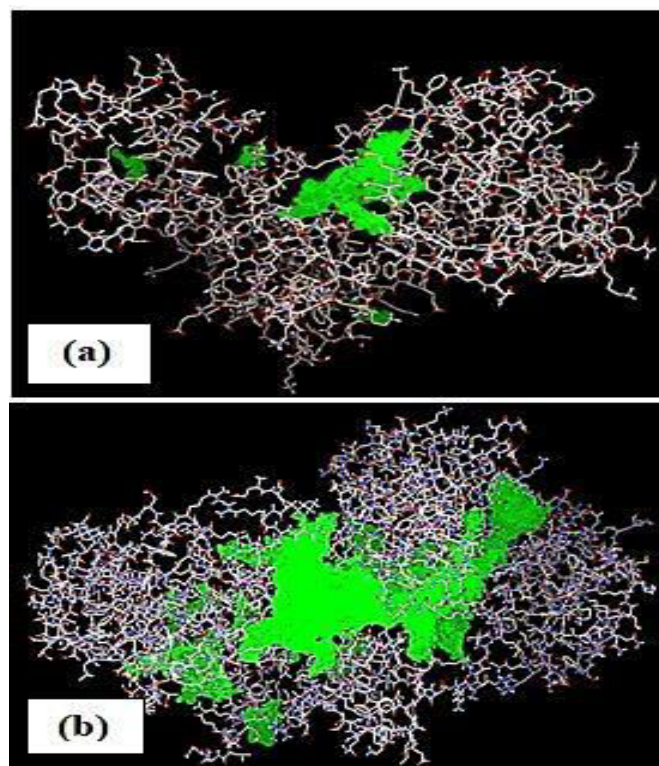


Fig. 1: Targets after preparation

The targets were processed in BIOVIA discovery studio by eliminating the water molecules and Hetatms from the targets and replacing them with polar hydrogen after downloading the 3D structure from Protein Data Bank. Following that, the protein was further prepared in Molegro virtual docker by repairing or rebuilding protein residues, forming a surface, and detecting cavities for docking in the protein target independently (Fig.1). For ligand preparation, two softwares were used;

BIOVIA discovery studio and MVD. MVD was used to evaluate the Remdesivir and produced ligands against two target proteins. Following the completion of docking, the proper poses were determined automatically. The posture was then converted to a ligand, and the hydrogen interaction with the specific target was studied (Fig.3). Following this, protein labeling was done, and a cartoon depiction of the backbone was developed (Fig.2).

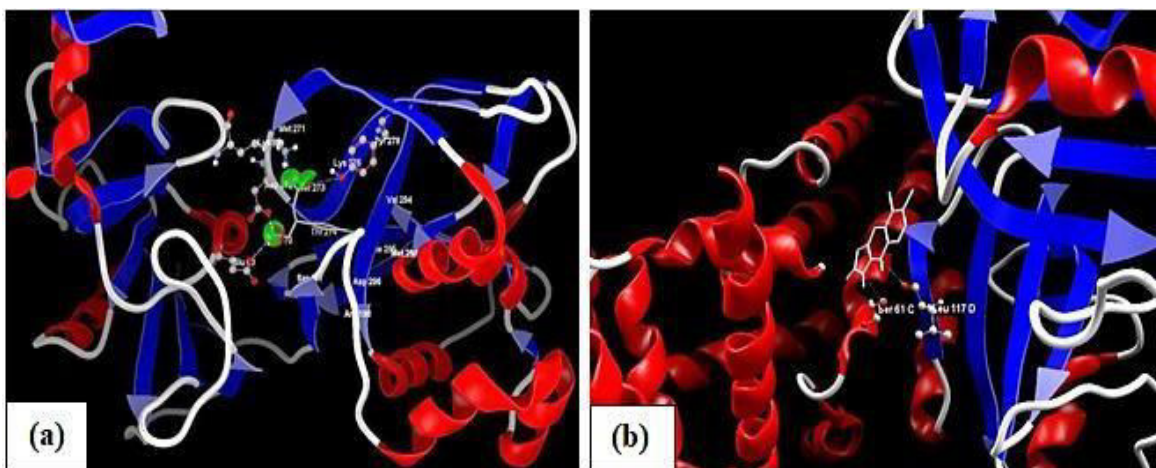


Fig. 2: Backbone creation for target protein (a) Nsp 15 and (b) Nsp 7 and 8 complex

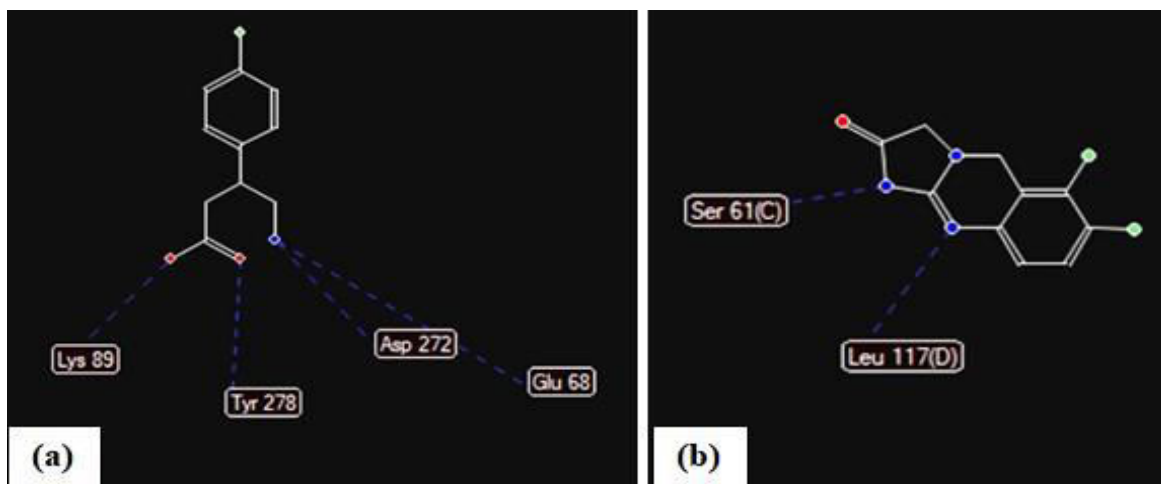


Fig. 3: Hydrogen bond interaction of ligands with amino acid residue of active site of (a) Nsp 15 and (b) Nsp 7 and 8 complex

Twenty compounds with the lowest energy value were chosen for future research from the docking output data (Table 1).

Cross validation was carried out using PyRx software. The reference compound Remdesivir and twenty selected compounds were docked with their respective targets again (Table 2).

The ADMET properties of the three drugs chosen for each target were examined. The Molinspiration online tool was used to analyze the ADMET features of the compounds in question (Table 3 and 4).

Remdesivir binding energy was found to be -176.677 kcal/mol for the Nsp 15 complex, -162.156 kcal/mol for the Nsp 7 and 8 complex. Out of 4,200 compounds,

twenty were chosen as targets because their binding energies were lower than Remdesivir's. The least energetic compounds were chosen for a reason: they would have less non-specific binding effects, and lower binding energy means the ligand will attach to the target with greater affinity. Molecules binding energy for the Nsp 15 target ranged from -240.782 kcal/mol to -179.676 kcal/mol, whereas molecules binding energy for the Nsp 7 and 8 complex ranged from -261.985 to -165.176 kcal/mol. Thereafter, the cross-validation was performed so that the results could be refined to a more

accurate level. In PyRx, docking Remdesivir to Nsp15 yielded -8.9 kcal/mol, while docking Nsp7 and 8 yielded -8.4 kcal/mol. The three drugs chosen for Nsp 15 were Danoprevir (-11.9 kcal/mol), Lanatoside C (-11.7 kcal/mol), and Digoxin (-10.4 kcal/mol), whereas Lanatoside C (-kcal/mol), Ledipasvir (-10.1 kcal/mol), and BMS-790052 (-9.8 kcal/mol) were chosen for Nsp 7 and 8. Molinspiration software is based on the Lipinski rule of five, which is used to evaluate drug similarity and features such as LogP, Molecular weight, hydrogen bond donor and acceptor, and Molar refractivity [23].

Table 1: Twenty identified lowest energy molecules for Nsp15 and Nsp 7 and 8 complex

Target	Name of molecules with Binding energy (KCAL/MOL)
Nsp 15	Remdesivir (-176.677), Birinapant (-240.782) , Danoprevir (-216.875), Myleran (-215.874), S5652 Elbasvir (-213.473), Cobicistat (-202. 678), Ritonavir (-202. 678), Docetaxel (-198.526), S5015 Simeprevir (-198. 176), Carfilzomib (-193.331), S9399 12346-O-Pentagalloylglucose (-192.791), Digoxin (-190.483), GMB-475 (- 189.858), Erythromycin ethylsuccinate (-187.308), S5554 Lanatoside C (-187.27), Gypenoside (-185.352), Norvir (-185.039), Vumon (-184.075), Lopinavir (-182.509), Valrubicin (-180.435), Oxybenzone (-179.676).
Nsp 7-8 complex	Remdesivir (-162.156), S9399 12346-O-Pentagalloylglucose (-261.985), Birinapant(-214.006), Ledipasvir (-196.681), S3724 Velpatasvir (-191.824), S554 Lanatoside C (-191.13), Cobicistat, Carfilzomib (-186.56), Elbasvir (-182.574), Docetaxel (-182.372), Sennoside A (-180.123), BMS-790052 (-175.184), Atazanavir Sulfate (-173.551), S3924 Ginsenoside Rb1 (-171.753), Danoprevir (-170.518), Azithromycin (-170.175), S5458 Verbascoside (-170.151), Fosamprenavir calcium salt (-166.986), S4935 Asunaprevir (-166.236), Glycyrrhizic acid (-165.887), Ritonavir (-165.176).

Table 2: Cross validation results for the Nsp 15 and Nsp7 and 8 complex target protein

Targets	Name of molecules with binding energy
Nsp 15	Remdesivir (-8.9), Danoprevir (-11.9), S5554 Lanatoside C (-11.7), Digoxin (-10.4).
Nsp 7-8 complex	Remdesivir (-8.4), Lanatocide C (-11.0), Ledipasvir (-10.1), BMS-790052 (-9.8).

Table 3: Calculation of molecular property of molecules for Nsp 15 and Nsp 7 and 8 complex

Target	Name of molecules and their ADMET properties
Nsp 15	<ul style="list-style-type: none"> • Danoprevir (LogP 4.65, MW 731.84, H bond acceptor 14, H bond donor 3) • Lanatoside C (LogP -0.10, MW 985.13, H bond acceptor 20, H bond donor 8) • Digoxin (LogP 1.12, MW 780.95, H bond acceptor 14, H bond donor 6).
Nsp 7-8complex	<ul style="list-style-type: none"> • Lanatoside C (LogP -0.10, MW 985.13, H bond acceptor 20, H bond donor 8) • Ledipasvir (LogP 9.21, MW 889.02, H bond acceptor 14, H bond donor 4) • BMS-790052 (LogP 7.77, MW 738.89, H bond acceptor 14, H bond donor 4).

Table 4: Calculation of Bioactivity scores of molecules for Nsp 15 and Nsp 7-8 complex

Receptors	Nsp 15			Nsp 7 and 8 complex		
	Danoprevir	Lanatoside C	Digoxin	Lanatoside C	Ledipasvir	BMS-790052
GPCR ligand	-0.05	-3.52	-1.28	-3.52	-2.94	-1.04
Ion channel modulator	-1.35	-3.68	-2.39	-3.68	-3.58	-2.17
Kinase inhibitor	-1.06	-3.72	-2.17	-3.72	-3.53	-1.71
Nuclear receptor	-1.15	-3.66	-1.88	-3.66	-3.62	-2.13
Protease inhibitor	0.72	-3.27	-0.88	-3.27	-2.24	-0.50
Enzyme inhibitor	-0.49	-3.33	-1.26	-3.33	-3.39	-1.48

The Lipinski rule of 5 applies if the molecule's molecular mass is less than 500 daltons, the hydrogen bond acceptor is less than 10, the hydrogen bond donor is less than 10, and logP is less than 5. The molecular properties of three lead compounds were determined as a result of this study. The results revealed that the lead compounds chosen for the Nsp 15 and Nsp 7 and 8 complexes did not obey the Lipinski rule of 5. This is because Lipinski's Rule of Five only applies to drugs taken orally, not antibiotics, injectable treatments, and so on. In case of bioactivity, active molecules can be identified from inactive ones based on their bioactivity. As long as the score is more than zero, the likelihood of the molecule demonstrating significant biological activity is the highest. Values in the range of 0.50 to 0.99 are regarded moderately active, while scores of 0.50 and lower are deemed inactive. Danoprevir was active against Protease inhibitors, according to the results of the bioactivity analysis. Neither Lanatoside C nor Digoxin received a -0.50 score. On the other hand, Lanatoside C, Ledipasvir, and BMS-790052 were discovered to be inactive compounds. As a result, Danoprevir, Lanatoside C, Digoxin, and Lanatoside C, Ledipasvir, BMS-790052 was identified as potential Nsp 15 and Nsp 7 and 8 complex inhibitors, respectively.

4. CONCLUSION

Current results have created an alternative paradigm for Danoprevir, Lanatoside C and Digoxin/ZINC242548690 as powerful inhibitors of Nsp 15 and Lanatoside C, Ledipasvir and BMS-790052 for Nsp 7 and 8 complex targets respectively. Additional analysis can be carried out to confirm their COVID-19 therapeutic potential.

Conflict of interest

None declared

Source of funding

None declared

5. REFERENCES

- Hui DS, I Azhar E, Madani TA, et al. *Int J Infect Dis*, 2020; **91**:264-266.
- Burki TK. Coronavirus in China. *Lancet Respir Med*, 2020; **8**(3):238.
- Liu J, Zheng X, Tong Q, et al. *J Med Virol*, 2020; **92**(5):491-494.
- Harapan H, Itoh N, Yufika A, et al. *J Infect Public Health*, 2020; **13**(5):667-673.
- Li Q, Guan X, Wu P, et al. *N Engl J Med*, 2020; **382**(13): 1199-1207.
- Rothe C, Schunk M, Sothmann P, et al. *N Engl J Med*, 2020; **382**(10):970-971.
- Baden LR, Rubin EJ. *N Engl J Med*, 2020; **382**(19): 1851-1852.
- Monteleone G, Sarzi-Puttini PC, Ardizzone S. *Lancet Rheumatol*, 2020; **2**(5): 255-256.
- Wu C, Liu Y, Yang Y, et al. *Acta Pharm Sin B*, 2020; **10**(5):766-788.
- Yang CW, Chen MF. *J Microbiol Immunol Infect*, 2020; **53**(3):419-424.
- Wang Y, Grunewald M, Perlman S. *Methods Mol Biol*, 2020; **2203**:1-29.
- Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y. *Mil Med Res*, 2020; **7**:1-10.
- Perlman S, Netland J. *Nat Rev Microbiol*, 2009; **7**(6):439-450.
- U.S. Food and Drug Administration. FDA Approves First Treatment for COVID-19. 2020.
- Yin W, Mao C, Luan X, et al. *Science*, 2020; **368**(6498):1499-1504.
- Chu CM, Cheng VC, Hung IF, et al. *Thorax*, 2004; **59**(3): 252-256.
- Xu Z, Peng C, Shi Y, et al. *bioRxiv*, 2020; 1-20.
- Shah B, Modi P, Sagar SR. *Life Sci*, 2020; **252**:1-12.
- Yang R, Liu H, Bai C, et al. *Pharmacol Res*, 2020; **157**:1-13.
- Koshak DAE, Koshak PEA. *Curr Ther Res Clin Exp*, 2020; **93**:1-3.
- Elfiky AA. *Life Sci*, 2020; **248**:1-6.
- Bansal N, Zheng Z, Song LF, Pei J, Merz KM JR. *J Am Chem Soc*, 2018; **140**(16):5434-5446.
- Kumar A, Choudhir G, Shukla SK, et al. *J Biomol Struct Dyn*, 2021; **39**(10):3760-3770.