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Vitamin D is a potential inhibitor of COVID-19: *In silico* molecular docking to the binding site of SARS-CoV-2 endoribonuclease Nsp15

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Abstract: Novel coronavirus disease (COVID-19) has become a pandemic threat to public health. Vaccines and targeted therapeutics to prevent infections and stop virus proliferation are currently lacking. Endoribonuclease Nsp15 plays a vital role in the life cycle, including replication and transcription as well as virulence of the virus. Here, we investigated Vitamin D for its *in silico* potential inhibition of the binding sites of SARS-CoV-2 endoribonuclease Nsp15. In this study, we selected Remdesivir, Chloroquine, Hydroxychloroquine and Vitamin D to study the potential binding affinity with the putative binding sites of endoribonuclease Nsp15 of COVID-19. The docking study was applied to rationalize the possible interactions of the target compounds with the active site of endoribonuclease Nsp 15. Among the results, Vitamin D was found to have the highest potency with strongest interaction in terms of LBE, lowest RMSD, and lowest inhibition intensity Ki than the other standard compounds. The investigation results of endoribonuclease Nsp15 on the PrankWeb server showed that there are three prospective binding sites with the ligands. The singularity of Vitamin D interaction with the three pockets, particularly in the second pocket, may write down Vitamin D as a potential inhibitor of COVID-19 Nsp15 endoribonuclease binding sites and favour addition of Vitamin D in the treatment plan for COVID-19 alone or in combination with the other used drugs in this purpose, which deserves exploration in further *in vitro* and *in vivo* studies.

Keywords: Vitamin D; COVID-19; SARS-CoV-2, endoribonuclease Nsp15, docking studies, pharmaceutics.

INTRODUCTION

The coronavirus disease (COVID-19), caused by the novel coronavirus SARS-CoV-2, has become the current health concern to the entire world (Saber-Ayad, Saleh *et al*., 2020, Shah, Modi *et al*., 2020). Unfortunately, there are no specific drugs or vaccines available to control the symptoms or the spread of this disease. SARS-CoV-2 belongs to the *Sarbecovirus* subgenus (genus *Betacoronavirus*, family *Coronaviridae*) together with SARS-CoV that emerged in 2002 causing ~8000 infections with a lethality of 10% (Jin, Zhao *et al*., 2020). The SARS-CoV-2 is an enveloped single-stranded, positive-strand ribonucleic acid (RNA) beta-coronavirus that has an ~30,000 nt RNA genome (Jin, Zhao *et al*., 2020). The genome contains a large replicase gene encompassing nonstructural proteins (Nsps), followed by structural and accessory genes (Kim, Jedrzejczak *et al*., 2020). The replicase gene encodes two ORFs; the first open reading frame encodes two translational products, polyproteins 1a and 1ab (pp1a and pp1ab) which are processed into mature non-structural proteins by the main protease (Mpro) and a papain-like protease (Cui, Li *et al*., 2019).

Non-structural protein 15 (Nsp15) encoded by coronavirus is actually a nidoviral uridylate-specific endoribonuclease (NendoU) that is usually highly conserved among vertebrate nidoviruses (coronaviruses and arteriviruses) and plays a vital role in the life cycle including replication and transcription as well as virulence of such viruses (Deng, Hackbart *et al*., 2017, Liu, Fang *et al*., 2019). Nsp15 is an IFN antagonist and it suppresses production of interferon-β by means of an endoribonuclease activity (Zhang, Li *et al*., 2018). Typically, the protein Nsp15 is a significant 3'-5' exoribonuclease which offers extra fidelity to the particular replication process. The exoribonuclease provides a proofreading operation to the complex which is often lacked by the RNA-dependent RNA polymerase. Proportionally, nsp7 and nsp8 proteins compose a hexadecameric sliding clamp as part of the complex which extremely augment the function of the RNA-dependent RNA polymerase (Fehr and Perlman, 2015). Nevertheless, the role of Nsp15 in coronavirus replication was ambiguous as EndoU-deficient coronaviruses were viable and replicated to near wild-type virus levels in fibroblasts. Moreover, EndoU facilitates the avoidance of viral double-stranded RNA identification by host macrophages (Deng and Baker, 2018). This new discovery of Nsp15/EndoU or NendoU functionality opened windows for new options to explore how a viral EndoU participates to pathogenesis and monopolizing this enzyme for new medications and vaccine design against COVID-19 (Deng and Baker, 2018).

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Vitamin D is a secosteroid prohormone that perform a substantial role in enhancement of several macro- and micro-minerals like calcium, phosphate, magnesium, zinc and iron. In human being, the most important components of fat-soluble secosteroids are cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) (Friedl and Zitt, 2017, Holick, 2006). This secosteroid vitamin is a major player in promoting the secretion of endogenously important peptides with antimicrobial potentials like cathelicidin (Gunville, Mourani *et al*., 2013). The findings that vitamin D level is significantly correlated with the severity of lower respiratory tract infection in children (Jat, 2017) and the advantageous effect of its supplementation to protect against flu infection, may indicate its significant influence on the immune system (Shalayel, *et al*., 2018).

It has been found that vitamin D modulates the immune system and that increased vitamin D supplementation boosts the immune protective rate and withstands inflammatory processes (Cheng, *et al*., 2020). Moreover, there is a hypothesis that viruses affecting respiratory epithelial tissues alter the expression of vitamin D receptors and by this expressional modulation they can negatively impact the antiviral potential of exogenous vitamin D (Telcian, *et al*., 2017). In this context, vitamin D is regarded as a principal defensive player against viral infections including HIV-1 infection by promoting adaptive and innate immunity (Jiménez-Sousa *et al*., 2018).

Nowadays, *in silico* assay has begun to be a trend in the world of pharmaceutical and biological research because its ability to provide many features by saving time, energy and efforts (Mavromoustakos, Durdagi *et al*., 2011). Many *in silico* software are useful in drug discovery approaches, the most important of these is AutoDock Tools, which enables us to calculate the free binding energy and understand the mechanism of interaction between the complex of protein-ligand. Also, the software enable us to perform the molecular modeling, screening and view analyzed protein complex with the ligands such as Discovery Studio (Jamkhande, Ghante *et al*., 2017). A point often overlooked *in silico*, this point is the size of the binding site and type of amino acids that built up the pocket. A number of web services for binding site predictions have recently been developed. PrankWeb (http://prankweb.cz/) and DeepSite (https://www.playmolecule.org/deepsite) were the most common web services reported in the literature for the structural and sequence visual view of protein binding site (Jendele, Krivak *et al*., 2019, Jimenez, Doerr *et al*., 2017).

This study aimed to reveal the proposed anti-viral role and the mechanism of binding of Vitamin D to all prospective pockets inside the Nsp15 of COVID-19 and compare its Lowest Binding Energies (LBE) with that in some drugs like Chloroquine, Hydroxychloroquine, and Remdesivir which are newly utilized for treatment of COVID-19.

MATERIALS AND METHODS

Protein preparation

The crystal structure of endoribonuclease Nsp15 was downloaded from the Protein Data Bank database (PDB ID: 6VWW) (Kim, Jedrzejczak *et al*., 2020). The protein preparation protocol was performed to prepare the enzyme using BIOVIA Discovery Studio Visualizer 16.1. Then, geometry optimization and energy minimization of endoribonuclease was conducted by removing H2O molecules. The protonation of endoribonuclease was done through "Protonate 3D" feature.

Ligands preparation

Based on recent clinical studies and literature reviews, we selected Remdesivir, Chloroquine, Hydroxychloroquine, and Vitamin D to study the potential binding affinity with the putative binding sites of endoribonuclease Nsp15 in COVID-19 (Cao, Deng *et al*., 2020, Colson, Rolain *et al*., 2020, Ferner and Aronson, 2020, Grant, Lahore *et al*., 2020, Panarese and Shahini, 2020, Wang, Cao *et al*., 2020). The 2D chemical structure of ligands built using PerkinElmer ChemDraw software 16.0.0.82. Then, the sketched ligands subjected to energy minimization (MM2 force field) using PerkinElmer Chem3D 16.0 and saved in PDB format.

Binding site determination

The PrankWeb and DeepSite are machine learning web servers that predicts ligand-binding sites as a combination of the protein 3D structure, sequence, binding pocket lists, and their immediate visual analysis. Both of these are able to predict new binding sites and work directly with multi-domain protein structures (Jimenez, Doerr *et al*., 2017, Krivak and Hoksza, 2018). In this analysis, the main aim of using these two servers was to check the binding site coordinate and specify the amino acids in endoribonuclease Nsp15 pockets to confirm the findings. Firstly, we uploaded 6VWW.PDB to webservers (PrankWeb, DeepSite) and pressed submit. After that, the servers sent the submitted PDB to the pipeline server, to start in the prediction. Then, the pipeline server provided us with a URL address for the progression of the prediction process and tracking the results.

Molecular docking

The simulation of molecular docking was used to measure the binding affinity of the chemical functional groups of the ligand with amino acids in the binding site of endoribonuclease Nsp15 (Morris, Huey *et al*., 2009). Auto Dock 4.2.6 was used to simulate the docking process rely on click by click protocol (Rizvi, Shakil *et al*., 2013). Initially, we added polar hydrogen and

Kollman to endoribonuclease Nsp15, then saved as PDBQT. Gasteiger charges for the selected drugs were computed and saved also in PDBQT format. Grid box size was set to 50*50*50 for the three predicted pockets. The first pocket coordinate (as x, y, z respectively) was set to -94.65, 19.58, and -28.99. The second pocket was -78.80, 26.99, and -27.95. For the third site was set to -65.46, 35.32, and -16.39. The spacing of 0.375 Å. Genetics algorithm run was set to 150, while remaining parameters were kept as default. The docking log files were analyzed using AutoDock Tools (ADT) to find out docking score and the inhibitory constant (Ki) for the ligands with each prospective pocket and saved in PDBQT format. Then, we used BIOVIA Discovery Studio Visualizer 16.1 to illustrate 2D docked visualization analysis of the ligands with the amino acids inside the pockets (Khaerunnisa, Kurniawan *et al*., 2020).

STATISTICAL ANALYSIS

Auto Dock version 4.2.6 was used to simulate the docking process.In addition, BIOVIA Discovery Studio Visualizer 16.1 was used to show the docked visualization analysis.

RESULTS

Binding site determination

The investigation results of endoribonuclease Nsp15 on the PrankWeb server showed that there are three prospective binding sites with the ligands as presented in fig. 1.

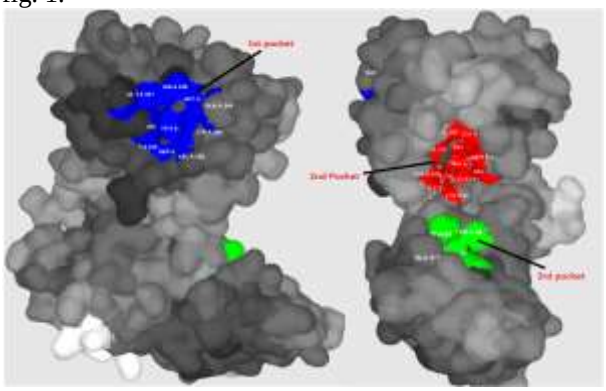

Fig. 1: Prank Web prediction of endoribonuclease Nsp15. The first pocket is coloured in blue. The second is in red, and the third is in green.

The first binding site is the highest pocket score of all three pockets, with a score value 5.7832. This pocket consists of 12 amino acids that built up the pocket. For the second pocket, it was made up of 15 amino acids and had a pocket score 4.7232, which possesses an affinity to bind relatively as the first site (fig. 2). While the third pocket is the smallest cavity (7 amino acids) and the lowest affinity f binding (2.9251) compared with the 1st and 2nd sites. Table 1 shows the grid center of the three pocket sites and the types of amino acids that make up the pocket.

Molecular docking

The present study mainly centered on the pharmacophore-based virtual data bank screening, molecular docking and drug-similarity profiling.

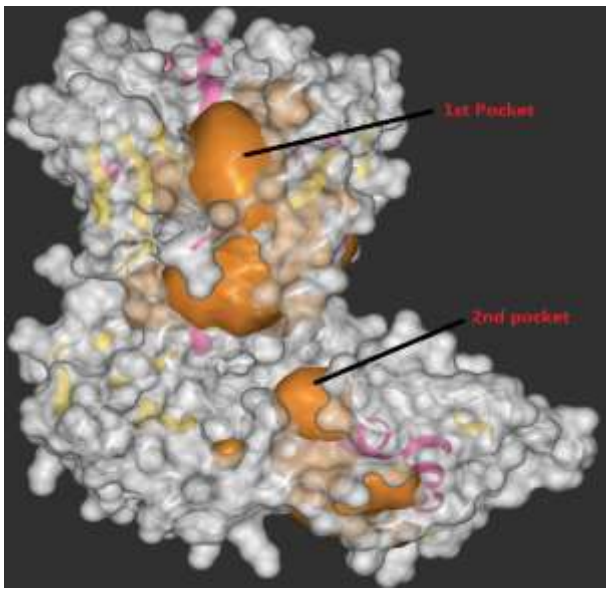

Fig. 2: Deep Site predictions of endoribonuclease Nsp15 in COVID-19 (6VWW.PDB). Where the pockets are colored in brown.

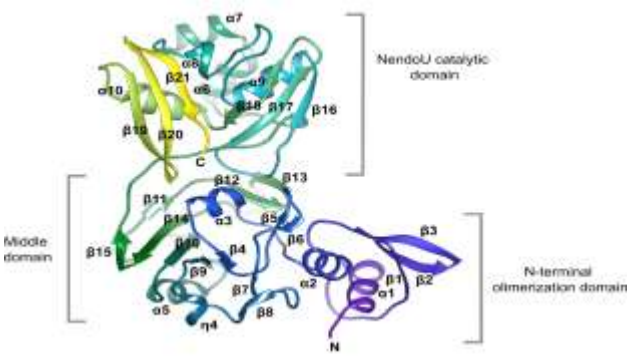

Fig. 3: The new crystal structure of Nsp15 Endoribonuclease (6VWW) from SARS CoV-2.

Here is the recent methodology and found out hit compound which strongly bound with the Nsp15 endoribonuclease of SARS-CoV-2 catalytic sites by blocking its proteolytic activity and maybe looked at as drug compounds. In our study, the Remdesivir, Chloroquine, Hydroxychloroquine, Vitamin D were docked to all three prospective binding sites of Nsp15 Endoribonuclease (6VWW. PDB) and the docking scores were presented in Table 2. The Chloroquine and Remdesivir are used as a control drugs to compare the docking results with Vitamin D and Hydroxychloroquine. Table 2 shows AutoDock 4.2 scores of the binding energy for the selected drugs with the three predicted binding sites in Nsp15 endoribonuclease of COVID-19.

Table 1: PrankWeb result summary of endoribonuclease Nsp15 of the pockets and the expected amino acids.

Pocket	Amino acids make up the pocket	Grid center		
		x	y	z
1st pocket	Polar amino acids: HIS235, GLN245, HIS250, LYS290, SER294 and THR341. Non-polar amino acids: LEU246, GLY247, VAL292, TRP333, TYR343 and PRO344.	-94.65	19.58	-28.99
2nd pocket	Polar amino acids: LYS90, THR196, SER198, ARG199, ASN200, ASP268, ASP273, SER274, THR275, LYS277 and ASP297. Non-polar amino acids: VAL70, LEU201, LEU252, LEU266, MET272, TYR279 and VAL295.	-78.80	26.99	-27.95
3rd pocket	Polar amino acids: GLU45 and ASP92. Non-polar amino acids: LEU43, PHE44, TRP59, TRP87 and TYR89.	65.46	35.32	-16.39

Furthermore, DeepSite server showed that there are two pockets inside the endoribonuclease Nsp15 (Fig. 2). DeepSite found that the first pocket coordinate was -74.41, 26.75, and -25.04 whereas; the second site was -56.41, 26.75, and -13.04.

Table 2: Molecular docking results of selected compounds with Nsp15 Endoribonuclease of COVID-19

Ligand Name	LBE Score with the 1st pocket (kcal/mol)	Ki (uM)	LBE Score with the 2nd pocket (kcal/mol)	Ki (uM)	LBE Score with the 3rd pocket (kcal/mol)	Ki (uM)
Remdesivir	-8.33	0.78	-7.97	1.44	-6.51	16.88
Chloroquine	-5.74	61.88	-7.23	5.01	-6.45	18.69
Hydroxychloroquine	-6.06	36.18	-7.00	7.42	-6.82	9.97
Vitamin D	-8.01	1.350	-10.26	0.030	-7.74	2.130

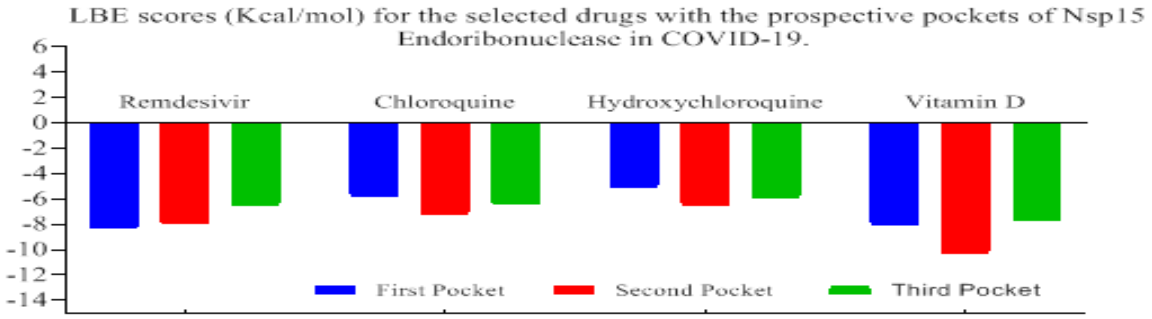

Fig. 4: Summary of Lowest Binding Energy (LBE) scores (kcal/mol) for Remdesivir, Chloroquine, Hydroxychloroquine and Vitamin D with the three predicted pockets

DISCUSSION

Prank Web permits fast visualization of results and gives valid predictions that could not be attained by other tools (Jendele *et al*., 2019). Many studies have been based on the fact that the endoribonuclease Nsp15 in COVID has no dominant binding site, and comparisons of the interactions with approved FDA drugs have also been performed without specifying a pocket position (Abou-Zeid, 2020, Gonzalez Paz, Lossada *et al*., 2020, Joshi and Poduri, 2020, Khan, Khan *et al*., 2020).

After analyzing Prank Web and Deep Site findings, we noticed both of the servers agreed that the endoribonuclease Nsp15 has more than one perspective binding region. The difference between the results of the machinery servers is that the PrankWeb foretell that there are three possible regions, while the DeepSite predicted the presence of two regions. When looking deeper into the outcomes, we find the DeepSite prophesy that the 1st and 2nd pockets which obtained from the PrankWeb are connected and forming a large pocket. The 3rd potential pocket of PrankWeb is mainly the second region reported by the DeepSite. This compatibility makes us sure that the pockets in endoribonuclease Nsp15 are more likely to be three possible regions as presented in fig. 1.

A recent study proved the relationship of SARS-CoV-2 with other beta coronaviruses on the amino acid level. The hyper-variable genomic hotspot has been built up in the SARS-CoV-2 populace at the nucleotide however not the amino acid level, proposing that there have been no significant changes. The transformations in nsp1, nsp3, nsp15 and gene S that right now distinguished is being related with the SARS-CoV-2 pandemic and was eligible for further researches (Wen, Yu *et al*., 2020). The predictions of virtual screening studies and binding energy calculations are generally more accurate if a high-resolution experimental structure of the target is available (Talluri, 2020).

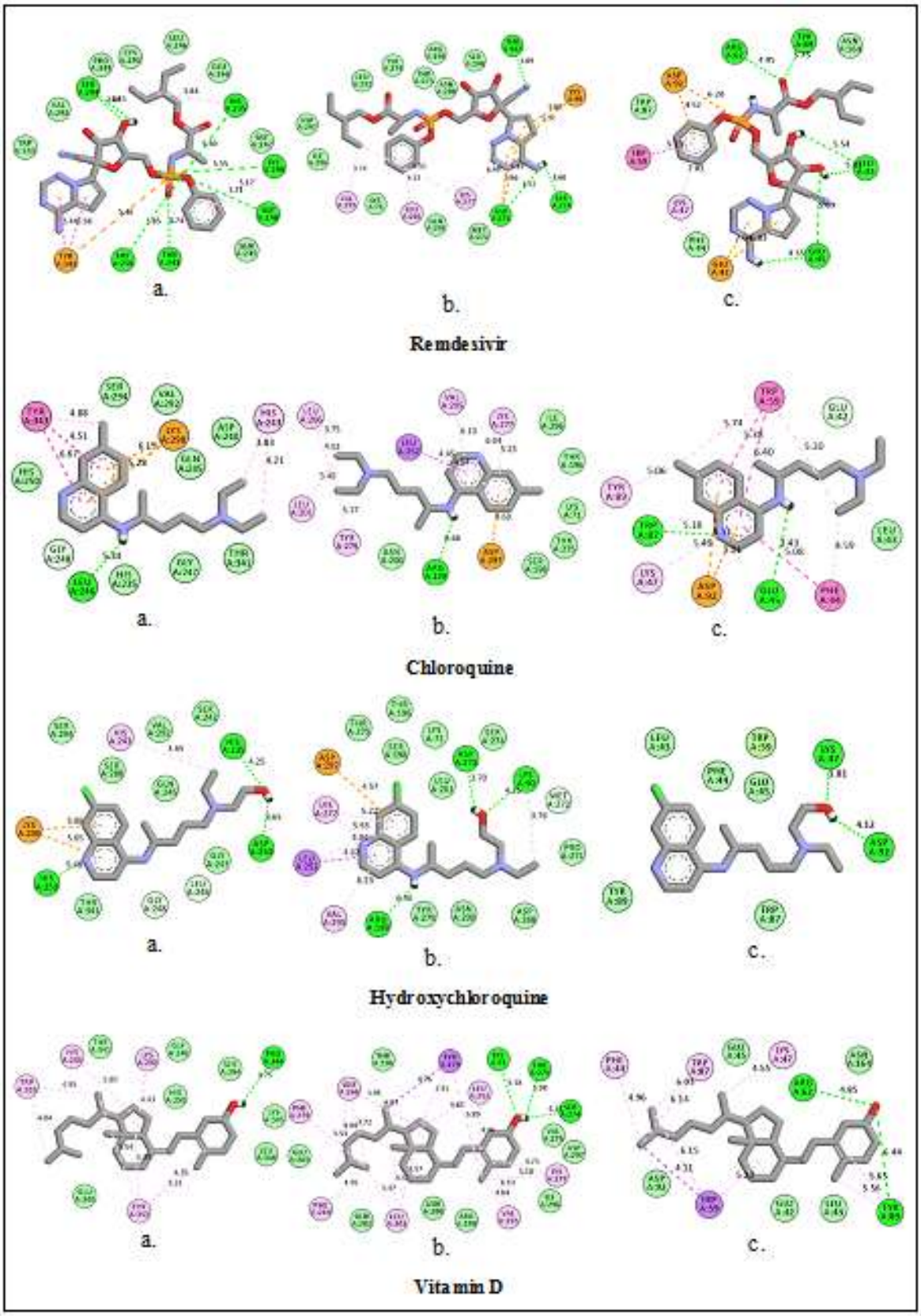

Fig. 5: 2D-interactions analysis of Remdesivir, Chloroquine, Hydroxychloroquine and Vitamin D with all pockets. a. 1st pocket, b. 2nd pocket and c. 3rd pocket by using BIOVIA Discovery Studio visualizer 16.1.

Recently, several docking experiments have been carried on the new crystal structure of corona virus Nsp15 (PDB code: 6VWW, fig. 3) to get a new therapeutic vaccine against SARS-CoV-2 but absolutely no drug or vaccine open to effectively combat this pathogen (Yu, Du *et al*., 2020). These studies tried to identify and validate useful lead compounds that could further be exploited and developed as candidate molecules that can progress to clinical trial. It is important to discover a cost-effective antiviral drug that could be used to control SARS-CoV-2. A competent way of drug discovery is to check whether existing drug-like compounds work against viral infections. The traditional drug discovery procedures are less efficient and time-taking (Hui, E *et al*., 2020). With regards to phylogeny, it seems almost certainly that the ancestor of the nowadays nidoviruses got its NendoU domain from the cellular homolog. The preservation of this kind of specific enzyme in the nidovirus replicase enhances the intricate legibility of the large viral replication machineries and proposes functional associations between nidoviral enzymes and cellular metabolic pathways, which needs further characterizations (Ricagno, Egloff *et al*., 2006).

Until now, the broad-spectrum antiviral drug Chloroquine is being used to take care of the SARS-CoV-2 in vitro (Wang, Cao *et al*., 2020). In silico research discovered that N3 inhibitor prevented the biological function of HCov-NL-63 by stopping its active catalytic site (Wang, Chen *et al*., 2016). In another recent study, Chloroquine phosphate has indicated better anti-SARS-CoV-2 activity; however, this medication has an obscure target of action. In docking results of a previous study, Chloroquine phosphate was expected to possibly combine with Nsp3b and E-channel (Wu, Liu *et al*., 2020), hence, observation of the anti-viral activity of this drug was evident by in vitro efficacy during culture tests on Vero E6 cells with 50% and 90% effective concentrations (EC50 and EC90 estimations) of 1.13 μM and 6.90 μM, respectively (Chen, Oezguen *et al*., 2016).

For the first potential site, the results exhibited a higher binding affinity for Remdesivir (-8.33 Kcal/mol) and Vitamin D (-8.01 Kcal/mol) compared to Chloroquine and Hydroxychloroquine. The binding affinity of Chloroquine and Hydroxychloroquine were about the same with the first site in term of the scores illustrated in table 2. Surprisingly in the second pocket, the docking score for Vitamin D was -10.26 kcal/mol and relatively the highest, followed by Remdesivir, Chloroquine, and Hydroxychloroquine, respectively. For the third pocket, the results were very close to each other with a distinctive binding affinity for Vitamin D, then Remdesivir > Hydroxychloroquine > Chloroquine. The docking score for the three pockets with (Remdesivir, Chloroquine, Hydroxychloroquine and Vitamin D) are shown more approachability in fig. 4. Our results showed that the docked scores of the Vitamin D with all three pockets were the best and with lowest binding energy, followed by Remdesivir, then Hydroxychloroquine, and finally Chloroquine.

Fig. 5 shows 2D interactions with all pockets for the selected drugs. Initially with the first pocket, the Remdesivir stuck strongly with this pocket by forming six hydrogen bonds with HIS235 (5.0 Å), GLY248 (3.71 Å), HIS250 (5.85 Å), LYS290 (5.55 Å), SER294 (2.83 Å), and THR34 (4.74 Å). It also made hydrophobic interactions with HIS235, LYS290 and TYR343. Amazingly, Vitamin D has formed a strong hydrogen bond with PRO344 at distance 4.75, as well as, Vitamin D created a map of hydrophobic interactions with HIS235, LYS290, TRP333 and TYR343. This positively enhanced the affinity of binding to the first site as Remdesivir. Relatively Hydroxychloroquine produced better bonds than Chloroquine, as it formed three hydrogen bonds with HIS235 (4.25 Å), APS240 (3.65 Å), and HIS250 (5.48 Å). While one hydrogen bond with LEU246 at a distance (5.34 Å) was found for Chloroquine. The scenario with the second pocket was unique, as Vitamin D emerged with a strong affinity for it, beating the rest drugs where it formed three strong hydrogen bonds with LYS71, SER274, and THR275 at distances 5.18 Å, 4.33 Å, and 3.98 Å, respectively. In addition, Vitamin D generated a spider map of hydrophobic interactions that affiliate stabilization inside the pocket. The findings were similar in the third pocket, with a preferential docked score for Vitamin D over the Hydroxychloroquine, Remdesivir, and Chloroquine, where Vitamin D formed two hydrogen bonds with ARG62 (4.85 Å), and TYR89 (6.44 Å). The singularity of Vitamin D within the three pockets, in particular in the second pocket, has been noted depending on the findings in fig. 5 and table 2 which enforces the possibility of using it as a potent inhibitor for Nsp15 of COVID-19 or in combination with Remdesivir, Chloroquine or Hydroxychloroquine.

CONCLUSION

The results of the present work revealed that Vitamin D may have potential to be used as a drug against COVID-19 more than Hydroxychloroquine and Chloroquine. Vitamin D showed the strongest interaction against the putative binding sites of the Nsp 15 of COVID-19 with respect to LBE than other compounds. The singularity of Vitamin D within the three pockets, particularly in the second pocket may favour addition of Vitamin D in treatment plan for COVID-19 alone or in combination with the other standard drugs is highly recommended, especially when we compare the relative adverse effects of these drugs with Vitamin D. In order to validate these docking outcomes and to understand real impact of Vitamin D, further *in vitro* and *in vivo* studies together with novel corona virus are strongly recommended.

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