



Vitamin D is a New Promising Inhibitor to the Main Protease (Mpro) of COVID-19 by Molecular Docking

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, vitamin D has shown greater efficacy of binding with M^{pro} of COVID-19 compared to the recently recommended drugs. The docking study was simulated to streamline interaction effects of Vitamin D, Remdesivir, Chloroquine, Hydroxychloroquine, Aspirin, and Azithromycin complexes with the active site of M^{pro}. Vitamin D is found to have the highest potential interaction in terms of total H-bond, van der Waal, torsional, and desolvation energy which were the lowest among all the selected drugs. The hydroxyl group of vitamin D and the thiol group of M^{pro} cysteine had played a leading role in increasing Vitamin D binding and stability with the M^{pro} pocket by contribution to the inception of three hydrogen bonds. The study recommend that vitamin D can be added to the COVID-19 treatment protocol, which may have the desired effect on viral replication inhibition and decreases mortality.

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1. INTRODUCTION

The world is now grappling with time to overcome the fight against COVID-19, which has no accepted treatment or procedure to deal with until now [1]. Despite being pronounced by WHO as a global pandemic disease, there are no absolutely approved vaccines and specific antiviral medications salutary to prohibit this viral infection. Hence, there is a great need to utilize some new compounds as potential antiviral candidates [2].

For multiplication, corona virus requires a group structural and non-structural proteins that are manufactured from polyproteins obtained by translation of its genomic RNA. These polyproteins are converted into structural and non-structural proteins mainly by the main protease (M^{pro}).

When the viral protease had been recognized as an appealing target for the inhibition of COVID-19 replication, many investigators tried to study the efficacy of some drugs for targeting such protease. Thus, the main protease, the so-called Mpro ($3CL^{pro}$) was acknowledged as an optimistic target among coronaviruses since it is preeminently implicated in the processing of viral polyproteins after being translated from the viral RNA [3,4]. During life cycle and replication of SARS-CoV-2 virus, the non-structural proteins comprising the main protease; M^{pro} (3-chymotrypsin-like protease, $3CL^{pro}$), RNA-dependent RNA polymerase, helicase, and, papain-like protease (PL^{pro}) play decisive function in this regard [5].

The M^{pro} is fundamental for survival of the SARS-CoV-2 virus as it is engaged at more or less 11 cleavage sites for the consistence of the different non-structural proteins that have great role in virus replication and transcription. In addition, the pp1a and pp1ab polyproteins in 16 different non-structural proteins are also processed by M^{pro} . These non-structural proteins are involved in the construction of subgenomic RNAs encoding four main structural proteins namely spike (S), nucleocapsid protein (N), envelope (E), and membrane (M) as well as the other accessory proteins [6].

It is important to use rational drug design approaches to assist laboratory researchers and guide them to choose the best among thousands

of medications that have been developed over the years. In order to save valuable time, energy, and reduce the mortality rate [7]. One of the most important rational drug design tools based on our known of the x-ray structure of the protein and the chemical structure of drugs is a computational molecular docking technique. A computational molecular docking technique is one of the most important rational tools used to determine the binding affinity between the protein and drug chemical structure [8,9].

Clinical data had displayed that vitamin D has a preventative impact against respiratory tract infections, and meta-analyses corroborated the correlation between the incidence of influenza and low 25(OH)D levels [10,11]. It is progressively realized that localized formation of vitamin D is accountable for numerous immune effects in respiratory diseases and may help in restraining SARS-CoV-2 infection [12,13].

In our previous in silico study, Vitamin D displayed, with respect to lower binding energy (LBE), the strongest interaction against the virtual binding sites of the Nsp15 of COVID-19 compared with the other drugs utilized for treatment of COVID-19 [14].

This research aimed to study the binding interaction of Vitamin D with the main protease (M^{pro}), which plays a key role in COVID-19 replication and compare that to the lately proposed drugs in COVID-19 treatment, such as Remdesivir, Chloroquine, Hydroxychloroquine, Aspirin, and Azithromycin.

2. METHODOLOGY

The X-ray crystal structure of the main protease was downloaded from the RCSB database (PDB ID: 6LU7) [15]. Biovia Discovery Studio Visualizer 16.1 was utilized to remove the heteroatoms, water, and prepare the protein further. The 2D chemical structures of Vitamin D and the supposed drugs (Remdesivir, Chloroquine, Hydroxychloroquine, Aspirin, and Azithromycin) were uploaded from the PubChem database. Then, PerkinElmer Chem3D 17.1 software was used to implement the MM2 force field at the ligands, and finally saved as PDB format.

AutoDock 4.2 is a computational software used to prepare the ligands and protein, as well as to

generate the docking process [16]. A click-by-click protocol was used to enforce this process [17]. Initially, the polar hydrogens and Kollman charge were added to the main protease. Then, we revitalized the selected drugs by Gasteiger charges. The size of the grid box was set to 50*50*50, and the coordinates were -10.244, 17.966, 66.508 (as x, y, z respectively) with spacing 0.375. For the docking parameter, the M^{PRO} was defined as rigid and drugs are flexible. The genetics algorithm run was set to 150, and the Lamarckian genetic was selection to proceed the docking, while the remaining parameters were kept default [17].

3. RESULT

3.1 Molecular Docking with M^{PRO}

Vitamin D, Remdesivir, Chloroquine, Hydroxychloroquine, Aspirin, and Azithromycin were docked to M^{PRO}. The predicted free binding energy (F.B.E), inhibition constant (Ki), van der Waals and H-bond desolvation energy, final intermolecular energy, torsional free energy, and unbound energy of Vitamin D and the selected drugs were given in Table 1.

As expected, all the selected ligands entered M^{PRO} pocket and were possess varying scores with the enclosed amino acids according to the

determined coordinates. Remarkably from Table 1, Vitamin D overtook the rest drugs, by forming a strong interaction with the protease (-7.26 kcal/mol). Followed by the presence of Chloroquine, Remdesivir, Aspirin and Hydroxychloroquine close to each other inside the M^{PRO} binding site. Obviously, the affinity of binding of the azithromycin was the least of all selected drugs. Also found the total H-bond, van der Waal, and the desolvation energy for vitamin D was the lowest. Then pursued by Remdesivir, Azithromycin, Hydroxychloroquine, Chloroquine, and Aspirin. The findings too showed that the torsional energy between vitamin D and M^{PRO} was mostly the least of all. The best score of the selected drugs (Vitamin D) was analyzed using complex Biovia Discovery Visualizer 16.1, LigPlot+ 2.1, and LigandScout 4.3 as presented in Fig. 1.

The hydroxyl group of vitamin D and the thiol group of M^{PRO} cysteine had played a leading role in increasing Vitamin D binding and stability with the main protease pocket by being contributed to the inception of three hydrogen bonds. Two H-bond donors forming one pairing H-bonds with the oxygen atom coded OG1 in THR24 and THR25 THR24, THR25 at distance 2.90 Å, and 2.75 Å respectively. And the third H-bond was acceptor from the oxygen atom (OG1) of THR45 with length 2.94 Å.

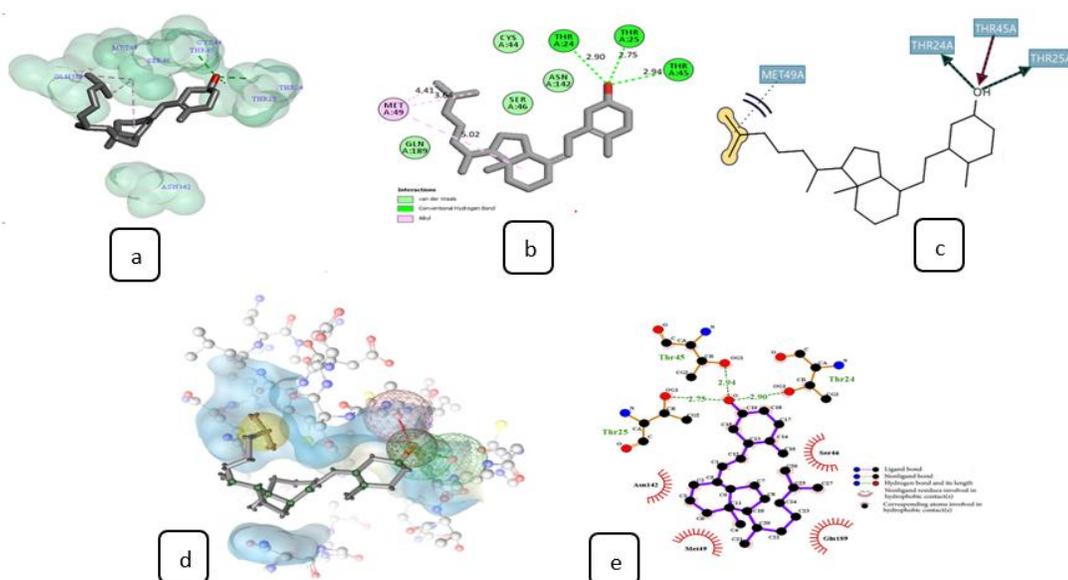


Fig. 1. 2D and 3D interactions models of Vitamin D complex with the active binding site of M^{PRO}. (a and b) were generated by using BIOVIA Discovery Studio visualizer 16.1. (c and d) by LigandScout 4.3. And (e) by LigPlot+2.1

Table 1. Free Binding Energy (F.B.E), inhibition constant (Ki), van der Waals and H-bond desolvation energy, electrostatic energy, and torsional free energy of all the chosen drugs with the M^{pro}

Compound	F.B.E (kcal/mole)	Ki (uM)	vdW + Hbond + desolv Energy (kcal/mole)	Electrostatic Energy (kcal/mole)	Torsional Free Energy (kcal/mole)
Remdesivir	-4.59	431.87	-9.06	-0.01	+5.07
Chloroquine	-4.68	373.83	-6.92	-0.14	+2.39
Hydroxychloroquine	-4.22	812.81	-7.06	-0.14	+2.98
Vitamin D	-7.26	4.76	-9.89	-0.06	+2.09
Aspirin	-4.46	533.64	-5.60	-0.06	+1.19
Azithromycin	-2.61	12.24	-8.70	-2.70	+8.35

Meanwhile, MET49 amino acid created a map of hydrophobic interactions with hexane ring and vitamin D alkyl chain that increased stability.

4. DISCUSSION

Presently, Coronavirus has turned out to be a major challenge for all the world. The outbreak of this particular virus is disseminating worldwide and leading to sundry mortalities. Nevertheless, simply no drugs are efficient for the avoidance of this pandemic. Thus, many researchers tried to find some natural products that might be useful to halt the spread of Coronavirus and simultaneously consolidate immunity [2]. So far, the broad-spectrum antiviral drugs including Chloroquine and Remdesivir are being *in vitro* utilized to beat SARS-CoV-2 [18]. Beating M^{pro} could be efficacious as it might cease the replication of SARS-CoV-2 RNA whereas suppressing NSP15 may bring into some structural changes which may never allow this virus to invade the particular host cells [19,20]. M^{pro} is a sound drug target for the virus as it plays an indispensable role in the typical viral replication and transcription. With this regard many *in silico* studies were conducted to show and evaluate the efficacy of some active ingredients like flavonoids and some other natural products against SARS-CoV-2 M^{pro} utilizing molecular docking technique. Taking in consideration the relative safe profile of these natural ingredients, these compounds could be held in future as an outset for new therapeutics against COVID-19 [6,21]. In our previous study on the efficacy of vitamin D as a potential inhibitor of SARS-CoV-2 endoribonuclease Nsp15, we found that Vitamin D uniquely suppressed the three active sites in Nsp15 with significant advantageousness when compared to the other drugs utilized in treatment of COVID-19 like Remdesivir, Chloroquine, and Hydroxychloroquine [20].

M^{pro} is a 33.8 kDa protein with 306 amino acid moieties that constituting three domains and COVID-19 virus M^{pro} comprises cysteine-histidine catalytic site and the active site of M^{pro} is composed of four sites (S10, S1, S2, and S4) that is quietly kept conserved in all the coronaviruses main protease. It has been formerly adduced that the substrate-binding site of SARS-CoV-2 M^{pro} is located in the cleft between Domain-I and II, and the 1 to 7 N-terminal amino acids residues are implicated in the proteolytic action. The C terminal domain III is typically globular cluster of 5 helices that regulates the salt-bridge interceded dimerization of M^{pro}. To maintain antiviral activity, the SH group of cysteine found in the S10 site readily anchors inhibitors [22,6].

It seems that the presence of thiol group found in cysteine moiety that anchors inhibitors of M^{pro} is fundamental for binding vitamin D to the binding site of the main protease since, cysteine is a main component of vitamin D binding proteins and vitamin D by itself inhibit cysteine proteases [23,24]. Continuously never before, in this research, a number of recently proposed drugs for the treatment of COVID-19 and vitamin D have been implemented to study their effect with the main protease binding site. The findings of the present study have shown that vitamin D was the best in forming bonds and can be used as a main or in combination with other drugs against COVID-19.

5. CONCLUSION

The results showed that vitamin D was highly capable of combining with M^{pro}, and the torsional energy was the lowest among the drugs chosen in this study. The hydroxyl group of vitamin D and the thiol group of M^{pro} cysteine had played a leading role in increasing Vitamin D binding and stability with the main protease pocket by being

contributed to the inception of three hydrogen bonds. The addition of Vitamin D to the COVID-19 treatment protocol may have a desired effect on the viral replication inhibition, enhancing immunity and reduce the mortality rate. To evaluate these core observations and understand the action of vitamin D, we highly recommend testing Vitamin D with COVID-19 in laboratory studies and *in vivo* assay.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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