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CORONAVIRUS AND ITS MAIN PROTEASE: AN INSIGHT FOR DRUGS DESIGN BY MOLECULAR DOCKING

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Abstract: Many viruses need their sulphydryl groups to be reduced in order to be allowed to enter cells. SARS-CoV-2, which belongs to *Coronaviridae* family and is responsible for coronavirus disease 2019 or COVID-19, has cysteine-rich proteins in its capsid as the main CoV protease (M^{PRO}), which must be intact and active maintaining the viral activity. Considering that M^{PRO} is an important molecular target for development of antiviral drugs, this work motivation was the structural study of the possible ways of interaction between drugs and viral cysteines by molecular docking technique for design of new potential inhibitors of M^{PRO} and its virulence.

Keywords: Coronavirus; Protease; Molecular docking.

1 INTRODUCTION

SARS-CoV-2, which emerged in Wuhan (China) is the "new coronavirus" responsible for COVID-19 (coronavirus disease). The initial cases of the disease have been attributed to the sale of live animals in Wuhan, which suggests a probable transmission of the virus from these animals to humans. Interpersonal contagion occurs through contaminated secretions, such as respiratory droplets, or contact with a contaminated surface. This easy transmissibility, worldwide, caused the global pandemic, as declared by the World Health Organization on March 11, 2020 (WHO, 2020), with an important morbimortality. To the present date (April 23, 2020) 213 countries have been affected, when 2,544,792 cases have been confirmed, with 175,694 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019).

China was the first country to adopt home isolation for tens of millions of

people, as a way of responding to the outbreak and trying to contain the spread of SARS-CoV-2. This virus has an incubation period of at least 14 days. In the first days, the analysis by RT-PCR (Reverse Transcriptase Polymerization Chain Reaction) is indicated, detecting fragments of the viral genetic material (RNA), when the patient's organism does not yet present important and detectable amounts of IgG and IgM anti SARS-CoV-2 (immune window). As for seroconversion, XIANG *et al.* (2020) in their assays managed to have the antibodies detected by ELISA (Enzyme-Linked Immunosorbent Assay) from the fourth day that the patients had symptoms. However, in view of the immunological window, ANVISA indicates this from the tenth day. Other techniques that analyze serology are immunochromatography, CLIA (Chemiluminescent Immunoassay) and immunofluoroscopy (XIANG *et al,* 2020; ANVISA, 2020).

According to a recent Chinese study, about 80% of patients had mild symptoms associated with the presence of the virus, such as dry cough, tiredness, fever (there may be a sore throat, nasal congestion or diarrhea) and recover from the disease without needing hospital treatment. The verified mortality rate was 2.3%, reaching 8.0% of patients aged between 70 and 79 years and 14.8% of those aged over 80 years (WU & MCGOOGAN, 2020). It is important to say that the large number of asymptomatic patients and the impossibility of testing the entire population are factors that underestimate this statistic, besides hindering the outbreak's control of coronavirus 2019.

On March 14 (2020), while China already had 84,215 cases, France faced COVID-19 with 4,420 infected cases. On the same date, Italy registered 20,207 cases, Spain 5,497 cases, Germany 3,755 cases, United Kingdom 1,010 cases, USA with 2,092 cases, Brazil with 119 (SANTÉ PUBLIQUE FRANCE, 2020; WHO, 2020).

Since it is evident the need for both treating symptomatic patients (increasing their survival), as well as for decreasing viral transmission, among the possible drugs for treating people with COVID-19, the use of old bioactive drugs, already elucidated by literature, it could be an interesting strategy, considering that the average time to a drug finally be sold as a medicine in a drugstore is 10 years and, at the end of this time, information such as safety profile, adverse effects, dosage and drug interactions must be well elucidated (COLSON *et al*., 2020).

An information about coronavirus which can be very useful for planning drugs with specific pharmacophoric groups and to interact with cysteine amino acids (Cys) is the fact that this virus has a considerable number of Cys in its main protease (M^{PRO}). Additionally, it is known that Cys residues are also abundant on the capsid of SARS-CoV-2, keeping it structured and conserved through disulfide bonds, favoring its interaction with healthy cells causing the transfer of viral RNA into them (ROWEN & ROBINS, 2020).

In March 2020, the three-dimensional structures of the SARS-CoV-2 M^{PRO} complexed with ligands "1 - [(2 ~ {S}) - 2-methylmorpholin-4-yl] -2-pyrazol-1-yl-ethanone ", derived from pyrazole and" N - [(5-methylisoxazol-3-yl) carbonyl] alanyl-L-valyl-N ~ 1 ~ - ((1R, 2Z) -4-(benzyloxy) -4-oxo-1 - {[(3R) -2-oxopyrrolidin-3-yl] methyl} but-2-enyl) -L-leucinamide ", derived from leucinamide, determined by the technique of crystallography and X-ray diffraction, were deposited on the Protein server Data Bank (https://www.rcsb.org/), by codes *5rf9* and *6lu7*.

Considering the relevance of this macromolecule as a molecular target to understand its possible inhibition by drugs already known at molecular level, the objective of this work was the study using molecular docking tools about interaction of M^{PRO} enzyme with: azidothymidine (AZT) (a), an important drug used for treatment of HIV positive patients and, in general, as antiretroviral (MAZUROV *et al*., 2010), with chloroquine (b) and with hydroxychloroquine (c), once recent studies have shown that these drugs have activity against SARS-CoV-2 (CORTEGIANI *et al*., 2020) with added vitamin D (d), which showed antiviral activity in a study previously published (BEARD *et al*., 2011). It is important to emphasize that, until the present date; there is no vaccine and / or treatment against COVID-19. Treatment with the use of plasma from immunized patients is not available to growing demands. So, social isolation is still the main strategy to prevent spreading and contamination by SARS-CoV-2 virus.

2 METHODOLOGY

Three-dimensional structures of ^{MPRO} from SARS-CoV-2 respectively complexed with pyrazole and leucinamide derivatives (codes 5rf9 and 6lu7) were retrieved from Protein Data Bank server (https://www.rcsb.org), renderized and visualized with the PyMol program (available for download at https://pymol.org) in order to investigate possible inhibition sites.

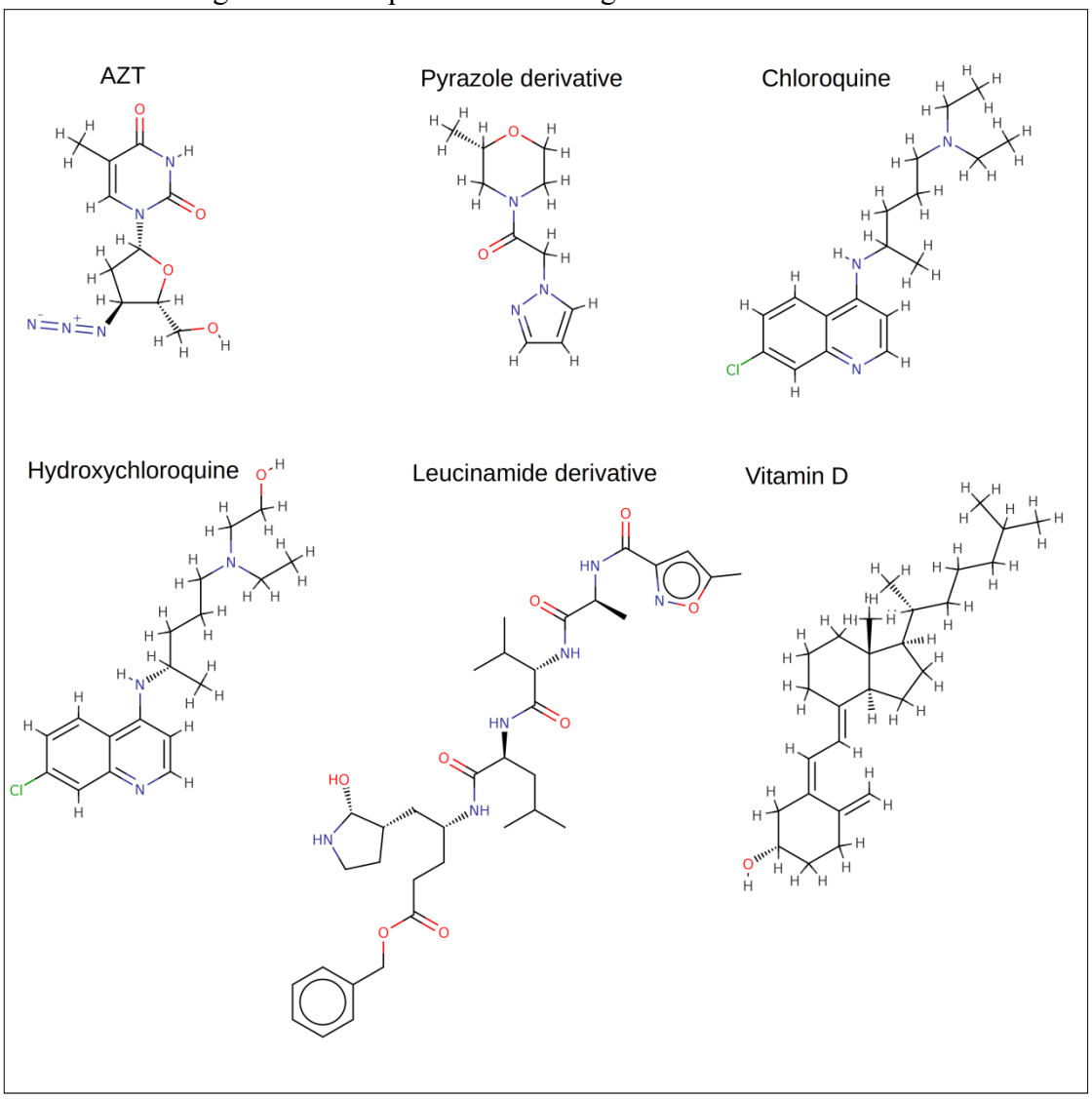
Otherwise, three-dimensional structures of all ligands used in this work, Figure 1, were prepared and optimized using classical mechanics, with the GHEMICAL program (HASSINEN & PERÄKYLÄ, 2001) and the TRIPES 5.2 force field (Shih & Chen, 1995). Subsequently, protonation status of each ligand at pH 7.4 was verified, using MARVINSKETCH program (free and available for download at: https://chemaxon.com/products/marvin) and then submitted to semi-empirical optimizations using the MOPAC 2016 program (STEWART, 2007) and the PM7 method (STEWART, 2007) to set them up with connection parameters, angles and dihedral angles as close as possible to experimental values.

After the semi-empirical optimizations, the three-dimensional structures of the ligands were submitted to ACPYPE for adding of AM1-BCC charges (DA SILVA & VRANKEN, 2012), where output files with extension *.mol2 were generated, recognized by AUTODOCK VINA, the program used in this work for molecular docking (TROTT & OLSON, 2010).

In a parallel manner, M^{PRO} of SARS-CoV-2 was separated from the inhibitor, visualized and prepared with the AUTODOCK TOOLS package (MORRIS *et al*., 2009), in which polar hydrogens were added, which was important for prediction of inter and intramolecular interactions by hydrogen bonds. Then, the interaction region of protein encompassing both active sites for binding to pyrazole derivative and to leucinamide derivative were considered to molecular docking and surrounded by a parallelepipedal box with micro boxes separated by 0.1 nm (small grid box) for the calculation of the potential surface, necessary for prediction of electrostatic receptor-ligand interactions. Thus, all needed parameters for carrying out the molecular docking were added to a text file (conf.txt), according to the following description: receiver = SARS-CoV-2.pdbqt; ligand = ligand.pdbqt; center_x = -19.293; center_y = 23.141; center_z = 66.114; size_x = 50; size_y = 52; size_z = 50; cpu = 4; num_modes = 20. As molecular docking is a stochastic technique, all calculations were performed 20 times, with extraction of the 20 best results for each ligand, totaling 400 results per ligand and 2400 conformers. Of the 400 conformers per ligand, the one

with the lowest interaction ΔG value was selected. That is, the more negative ΔG value, more favorable the receptor-ligand interaction in thermodynamic aspect (PIETRALONGA *et al.*, 2015). Molecular docking assays are basically receptor-ligand interactions simulated on the computer, where the ligand searches for electrostatic and van der Waals complementarities, considering the effect of the solvent is treated implicitly through dielectric constant, in addition to a sphere or box that encompasses the system of interest.

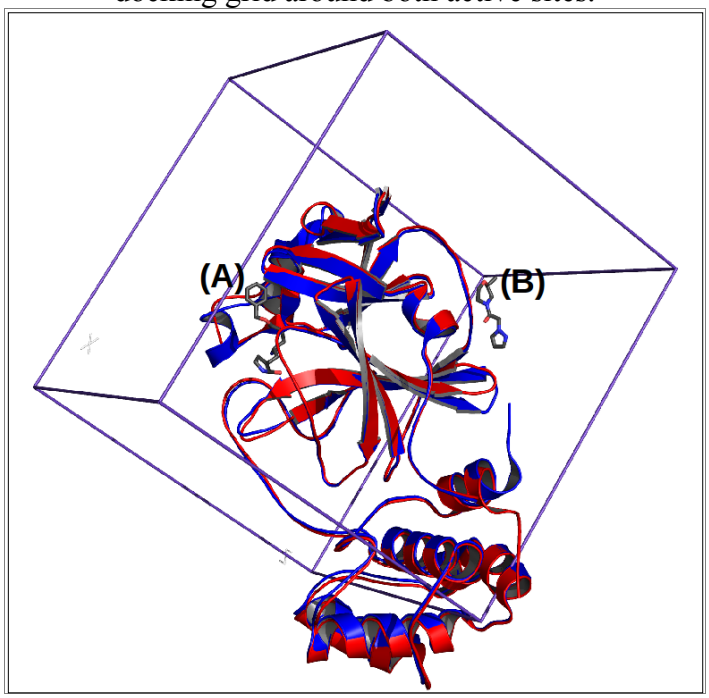
Figure 1: 2D Representation of ligands used in this work.



3 RESULTS AND DISCUSSION

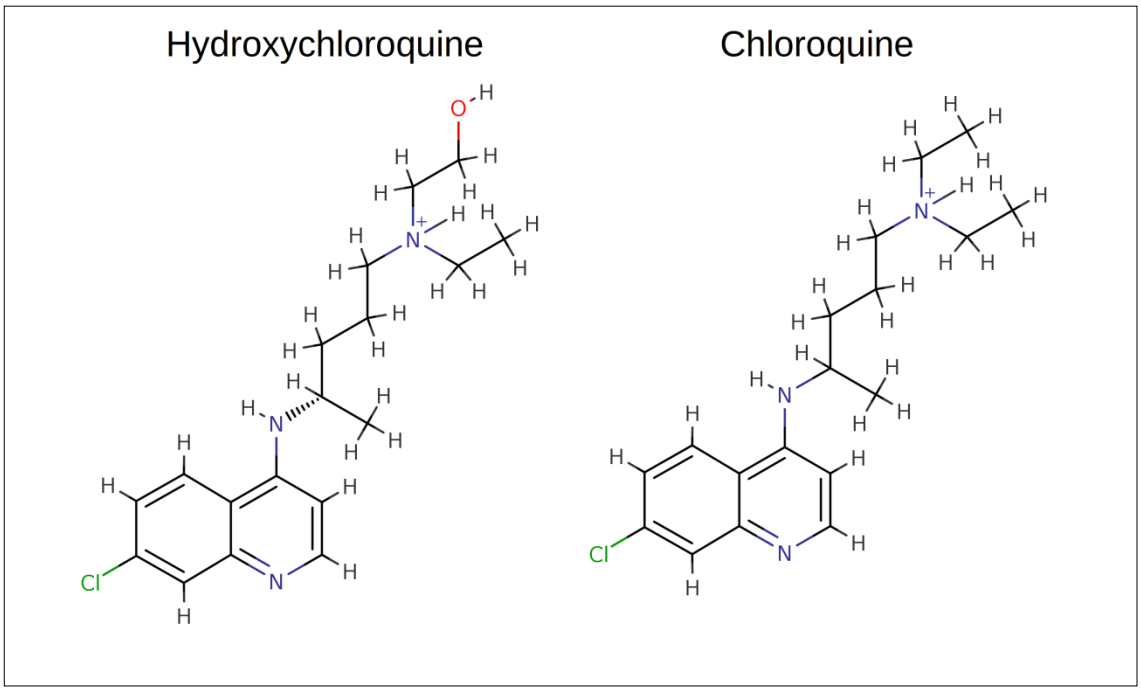
Initially, code structures 6lu7 and 5rf9 were opened and aligned with the PyMol program. The alignment showed SARS-CoV-2 M^{PRO} with two important inhibition sites, one to which the leucinamide derivative is attached, Figure 2 (A) and the other to the pyrazole derivative, Figure 2 (B). From this initial crystallographic information, proceeding the molecular docking simulations, if molecules used in this work interact more strongly or weakly to the binding sites of M^{PRO} comparing with crystallographic ligands, results may suggest greater or lesser inhibitory activities.

Figure 2: Superposition between PBD codes 6lu7 and 5rf9 and their respective crystallographic inhibitors (A) Leucinamide derivative and (B) pirazole derivative with a docking grid around both active sites.



After protonation states prediction at pH 7.4 using the MARVINSKETCH program, hydroxychloroquine and chloroquine became positively charged, Figure 3, while the other ligands remained neutral.

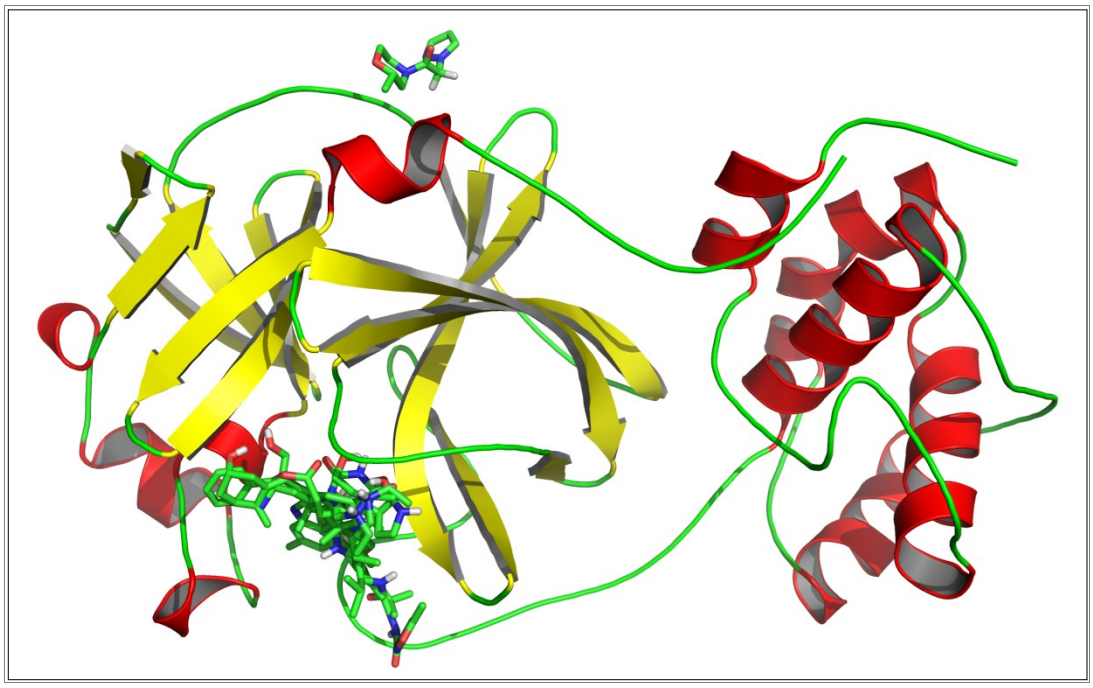
Figure 3: Protonation state of ligands after molecular treatment with MARVINSKETCH program.



After studying the protonation state of ligand molecules (pyrazole derivative, leucinamide derivative, AZT, chloroquine, hydroxychloroquine and vitamin D) at pH 7.4 (physiological condition), different systems formed by M^{PRO} from SARS-Cov-2 (receptor) + ligand were submitted to molecular docking simulations.

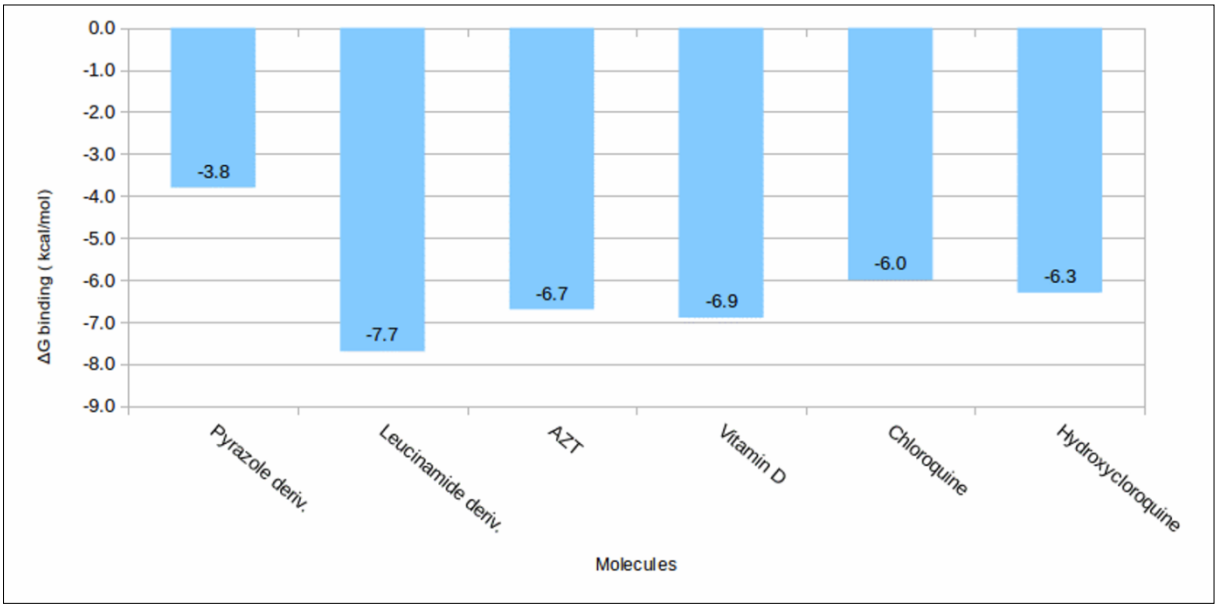
When analyzing the results of interactions between molecules with M^{PRO} from SARS-CoV-2, it was noted that except to pyrazole derivative ligand, all tested molecules interacted at the same binding site as the derived from leucinamide, Figure 4. The pyrazole derivative also showed affinity for a second binding site in this protease, corroborating the PDB data server, obtained by cristalographic and X-ray diffraction experiments. It is important to emphasize that binding of AZT, vitamin D, chloroquine and hydroxychloroquine molecules to the same region of active site from M^{PRO} enzyme of Coronavirus suggests that these compounds probably may have anti-viral activity. Our data may be compared with those obtained by clinical tests, recently carried out (data not published) in which some of the molecules used in an attempt to treat patients in serious conditions of COVID-19 are the same we used in our research. However, serious adverse effects were observed (JUURLINK, 2020; GAUTRET et al., 2020; TOURET et al., 2020). Regarding vitamin D, recent unpublished studies have shown that it may play an important role for treatment once patients with this syndrome showed a significant deficiency of this vitamin. It is worth to emphasize the importance of vitamin D for the body as a regulator of immune system (HANEL et al, 2020).

Figure 4: Superposition of all results of Molecular Docking with M^{PRO} from SARS-CoV-2.



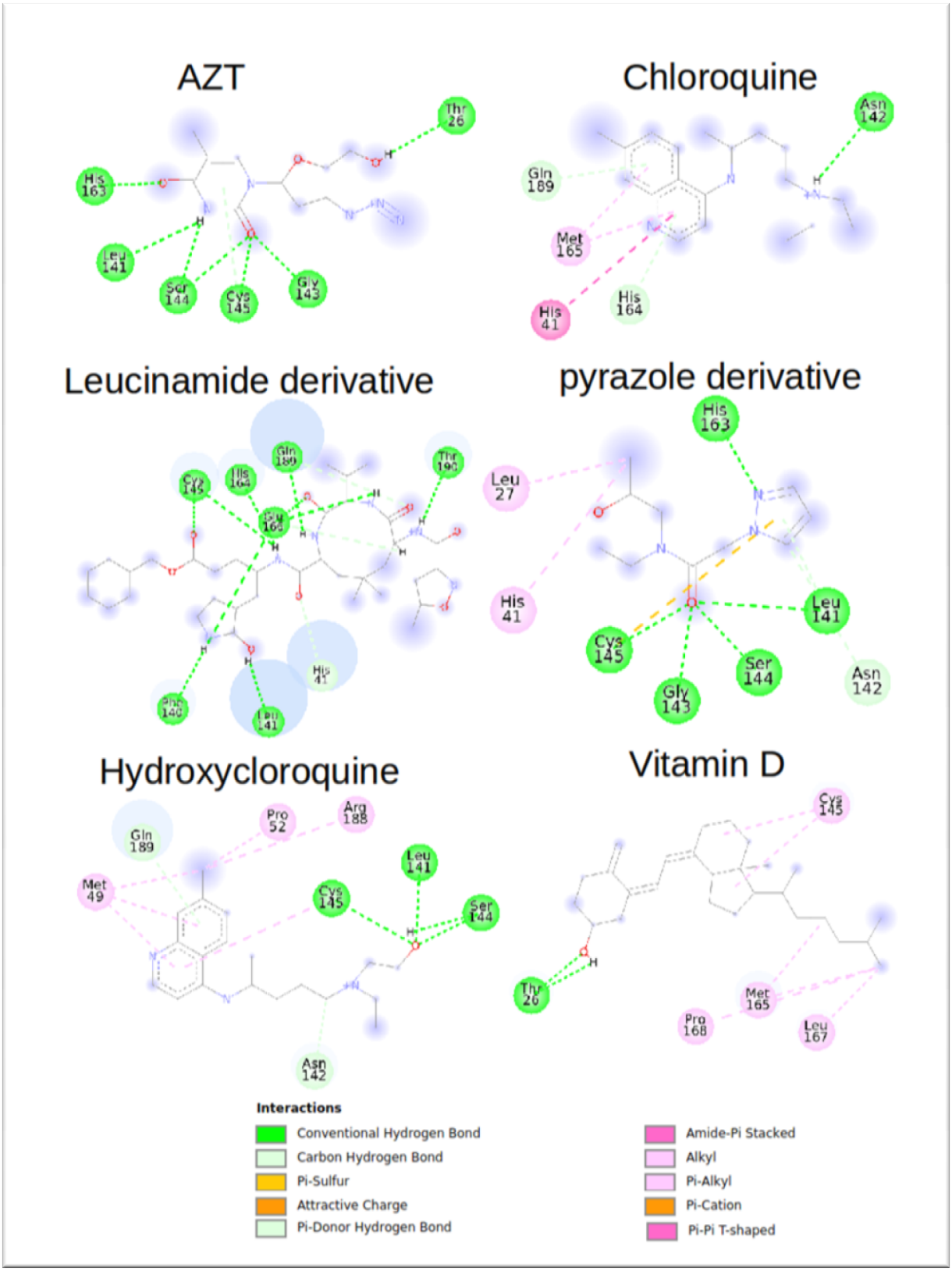
For monitoring interactions affinity between ligands molecules and the SARS-CoV-2 M^{PRO}, free binding energies were extracted and a graphic of $\Delta G_{binding}$ versus ligands was plotted, Figure 5, showing stronger linkages between M^{PRO} and leucinamide derivative (energy of -7.7 kcal / mol) in contrast to M^{PRO} and pyrazole derivative contacts (energy of -3.8 kcal / mol). For comparative analysis between each ligand and the leucinamide derivative, it is important to highlight that vitamin D showed the second best interaction energy (-6.9 kcal / mol), followed by AZT (-6.7 kcal / mol), hydroxychloroquine (-6.3 kcal / mol) and chloroquine (-6.0 kcal / mol). These data suggest that probably vitamin D may have a promising effect for the treatment of COVID-19. Also, we emphasize that vitamin D can be obtained from exposure to UVB radiation from sunlight and / or dietary supplements.

Figure 5: Free interaction energy *versus* ligands.



For better visualizing distinct types of interactions and the main amino acids in contact with MPRO, Figure 6 shows the 2D diagram of the interactions by molecular docking. We can see that all molecules, except chloroquine, interacted with Cys145 through hydrogen and Pi-Alkyl bonds, suggesting a possible inactivation of MPRO. This data corroborates the literature, describing Cys145 residue as one of the catalytic amino acids of MPRO of SARS-CoV-2 (ZHANG et al., 2020). In particular, vitamin D shows contact with Cys145 (predominantly by Pi-Alkyl stacking) and is strongly kept at the active site from protease via hydrogen bonding with its amino acid Thr26. In addition, several nonpolar contacts are observed between vitamin D and the enzymatic active site of MPRO, meaning a second best affinity energy value, considering that van der Waals contributions are such as important as electrostatic ones.

Figure 6: 2D visualization of present compounds with stronger interaction with amino acids from protease.



4 CONCLUSIONS

In the present work, the molecular docking method was used to study some promising molecules in treatment of COVID-19 disease via inhibition of the enzyme MPRO, the main protease of SARS-CoV-2. According to our molecular docking results, the ligands which showed the highest affinity between coronavirus MPRO was the leucinamide derivative, followed by vitamin D and AZT. Thus, it is possible to conclude that these last two ligands are the second and third best binding molecules respectively. Therefore, vitamin D, a virtually non-toxic molecule (up to 100 ng / mL in the blood) can be used for the treatment of COVID-19. Although the other molecules have reasonable interaction energies, it is important to highlight that clinical studies still need to be performed due to their adverse effects and their toxicity to mammals.

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