Virtual screening based on molecular docking of possible inhibitors of Covid-19 main protease

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ABSTRACT

Coronavirus (COVID-19) is an enveloped RNA virus that is diversely found in humans and that has now been declared a global pandemic by the World Health Organization. Thus, there is an urgent need to develop effective therapies and vaccines against this disease. In this context, this study aimed to evaluate *in silico* the molecular interactions of drugs with therapeutic indications for treatment of COVID-19 (Azithromycin, Baricitinib and Hydroxychloroquine) and drugs with similar structures (Chloroquine, Quinacrine and Ruxolitinib) in docking models from the SARS-CoV-2 main protease (M-pro) protein. The results showed that all inhibitors bound to the same enzyme site, more specifically in domain III of the SARS-CoV-2 main protease. Therefore, this study allows proposing the use of baricitinib and quinacrine, in combination with azithromycin; however, these computer simulations are just an initial step for conceiving new projects for the development of antiviral molecules.

1. Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2, a novel coronavirus from the same family as SARS-CoV and Middle East respiratory syndrome coronavirus (MERS), has spread worldwide, prompting the World Health Organization to declare a pandemic (Pascarella et al., 2020). CoVs are a large family of RNA viruses that are found in various animal species. They are known to cause diseases of the respiratory, hepatic, nervous and gastrointestinal systems in humans, with the potential to cause severe and possibly fatal infections [1,2].

The novel coronavirus uses the same receptor as SARS-CoV [angiotensin-converting enzyme 2 (ACE2)], and mainly spreads through the respiratory tract. Human-to-human aerosol transmission is undoubtedly the main source of contagion, which happens mainly through contaminated droplets, hands or surfaces [3].

The symptoms of COVID-19 infection appear after an incubation period of approximately 5.2 days, ranging from 6 to 41 days from the onset of COVID-19 symptoms to death [3]. This period seems to be associated with age, biological sex, and other health conditions, such as cardiovascular disease, hypertension, diabetes mellitus [1,4]. The complete clinical manifestation is not clear yet, but the reported symptoms range from mild to severe. Common symptoms include fever, cough, myalgia or fatigue, pneumonia, and increased dyspnea. The less common symptoms reported include headache, diarrhea, hemoptysis, runny nose, and phlegm-producing cough [1,5].

At present, there is no single specific antiviral therapy for COVID-19 and the main treatments are supportive [2]. However, drug repositioning has been a strategy adopted by several researchers to seek effective treatment in a short period. Besides that, a virtual screening based on molecular docking emerges as an important tool for obtaining new antiviral molecules, where researchers can use this tool as a complementary approach so that the synthesis of new compounds or the repositioning of drugs can be assigned [9].

While traditional methods of drug discovery can take years, the

approach taken here to search for possible medications for the SARS-CoV-2 is docking of models from the SARS-CoV-2 main protease (M-pro) protein [4]. This protein is a vital target for drug discovery studies against the recent coronavirus epidemics, including Severe Acute Respiratory Syndrome CoV (SARS) and MERS CoV [5].

Also, in the face of a global economic recession scenario, infrastructure requirements for experimental trials are out of reach for most researchers due to their high cost; thus, computational analysis allows that, through simulations, it is possible to develop new projects for the initial stages of discovering new antimicrobial agents [8,9]. Thus, this study aimed at evaluating the drugs' interaction with other therapeutic indications that have been described for the treatment of COVID-19 (Azithromycin, Baricitinib, and Hydroxychloroquine), and their similar structures (Chloroquine, Quinacrine, and Ruxolitinib) compared to the COVID-19 main protease in the complex through computer simulations.

2. Materials and methods

2.1. Enzyme collection and preparation

Using the data filed in the Protein Data Bank (https://www.rcsb.org/) database, the structure of the COVID-19 virus main protease (M^{pro}) was obtained, identified in the repository as *The crystal structure of COVID-19 main protease in complex with an inhibitor N3* PDB ID: 6LU7, composed of three domains, domain I (residue 8–101), domain II (102–184), domain III (201–303), and a long *loop* (185–200) binding domain II to domain III; its structure was filed in the Protein Data Bank with a resolution of 2.16 Å, determined from X-ray diffraction, classified as viral protein, *Bat SARS-like coronavirus* organism, and *Escherichia coli BL21(DE3)* expression system [6]. In the process of preparing the SARS-CoV-2 main protease, all residues were removed and polar hydrogens were added [7,8], producing favorable protonation states for molecular docking [9].

2.2. Binders collection and preparation

Redirecting approved drugs and drug candidates is an alternative approach to quickly identifying potential drugs to manage viral infections that arise quickly [6]. Thus, the chemical structures of the inhibitors Azithromycin (CID447043), Baricitinib (CID44205240), Chloroquine (CID2719), Hydroxychloroquine (CID3652), Quinacrine (CID237), and Ruxolitinib (CID25126798) were selected from the Pubchem repository (https://pubchem.ncbi.nlm.nih.gov/) (Fig. 1), and optimized from energy minimization protocol using the *steepest descente* algorithm, with cycles of 50 interactions and MMFF94 (Merck Molecular Force Field 94) force field [10,11], established in the Avogadro code (version 1.2.0) [12].

2.3. Molecular docking

The docking simulations between inhibitors and the SARS-CoV-2 main protease were performed using the AutoDock Vina code (version 1.1.2), employing 3-ways *multithreading, Lamarkian Genetic Algorithm* [13], and the feasible region center_x = −26.734, center_y = 13.009, center_z = 56.185, size_x = 94, size_y = 112, size_z = 108, spacing = 0.642, and exhaustiveness = 8. Between ten and forty molecular docking executions were performed, the most favorable ones being represented by the lowest free-bond energy (ΔG) [14], and number of simulations that were repeated in the same region of the biological receptor, i.e., preference of the molecule for the same bond site. Figures were drafted using the Discovery Studio Visualizer [15] and UCSF Chimera viewers [16].
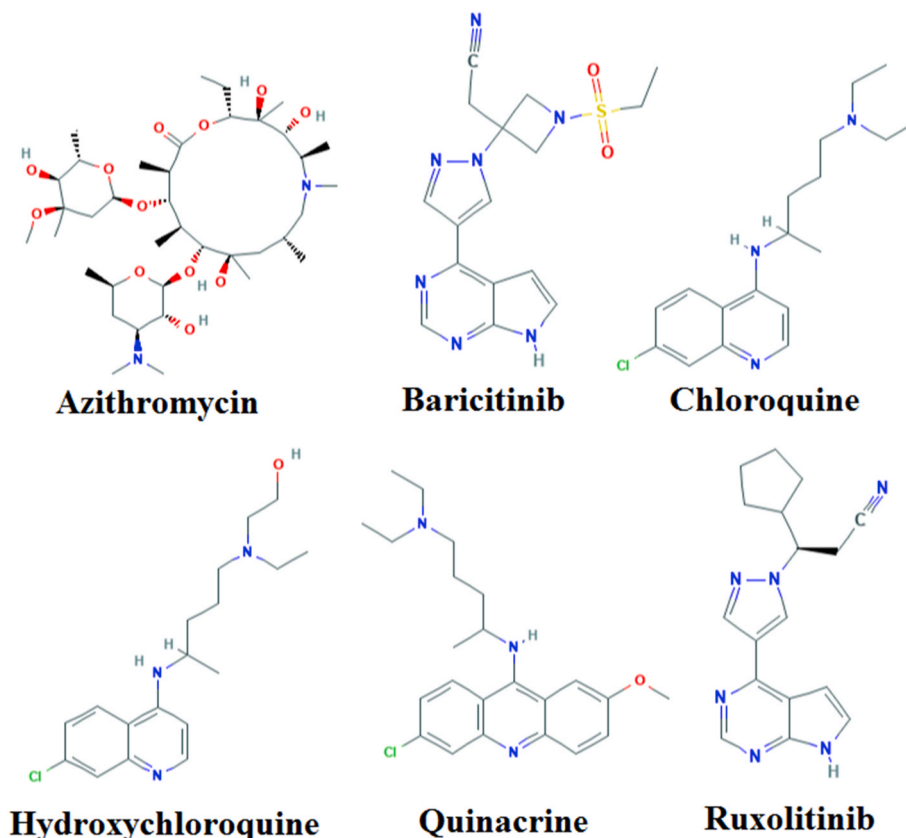

Fig. 1. Chemical structures of the ligands.

3. Results and discussion

For the understanding of receptor-binder interactions, the study of molecules employing molecular docking has become increasingly relevant to predict bond modes and elucidate experimental results [17]. Based on this, the molecular docking routines generated values of RMSD (*Root Mean Square Deviation)* [18] and bond-free energy [19] for complexes formed with variations in RMSD values from 1060 to 1978 Å, and bond-free energy of −6.6 to −4.7 kcal/mol (Table 1).

Analysis of the molecular anchoring simulations showed that all inhibitors are linked in the same enzyme site, more isolated in domain III of the main SARS-CoV-2 protease, but they are distant in the binding site of the N3 protease inhibitor, located between domains I and II (Fig. 2). Comparing the calculated distances between inhibitors and residues from the N3 binding site (Table 2), it was identified that all ligands were at a greater distance than the N3 complexed in the main protease of the COVID-19 virus (Mpro), highlighting what shows Fig. 2.

Analysis of the interactions showed that azithromycin (Fig. 3A) exhibited six interactions with COVID-19, one of the conventional hydrogen bond type with Leu272 (2.62 Å) and five of the alkyl type, two with Leu286, two with Leu287 and one with Met276. Although azithromycin showed a hydrogen bond with the enzyme, the inhibitor showed no interactions with the binding site compared to N3. Regarding the receptor-ligand complex formed with baricitinib (Fig. 3B), the ligand has ten interactions with the amino acid residues of the enzyme, three of the conventional hydrogen bond type; one with Lys137 (2.66 Å), one with Asp197 (2.42 Å) and one with Leu287 (2.48 Å); one van der Waals interaction with Thr199; two of the carbon hydrogen bond type, one with Leu287 and the other with Aps289; two interactions of the Pi-Cation type with Arg131; a Pi-Anion interaction with Asp289 and an interaction of the Amide-Pi stacked type with Thr198.

The chloroquine inhibitor (Fig. 3C) showed two interactions with the main protease of the COVID-19 virus, a conventional hydrogen bond with Tyr239 (1.92 Å) and a Pi-Pi T-Shaped type with Tyr237. The hydroxychloroquine (Fig. 3D) and chloroquine docking did not exhibit adequate binding energy with the enzyme (−5.0 kcal/mol), higher than the value of free binding energy considered in the literature as standard (−6.0 kcal/mol or less), which might have interfered with the interaction of hydroxychloroquine with the binding site of the N3 inhibitor complexed in the target enzyme. However, four interactions of hydroxychloroquine were found with the main SARS-CoV-2 protease, a Pi-Cation with Arg131, a Pi-Anion with Asp289 and two of the conventional hydrogen bond type, one with Lys137 (2.61 Å) and the other with Tyr237 (2.99 Å).

The quinacrine molecular docking routines (Fig. 3E) showed the formation of three interactions with the target protein, one of the conventional hydrogen bond type with Tyr239 (2.46 Å), one of the carbon hydrogen bond type with Thr199 and a Pi-Sigma with Leu272. Analysis of interactions with ruxolitinib showed that the molecular fitting simulation of the inhibitor (Fig. 3F) resulted in the formation of six interactions with the enzyme, one of the alkyl type with Leu287, a conventional hydrogen bond with Lys137 (3.07 Å), a carbon hydrogen bond with Leu287, an unfavorable acceptor-acceptor interaction with Tyr239 and a Pi-Anion with Asp289. Hydrogen bonds play an important role in macromolecular recognition, folding and stability [20].

Thus, the formation of nine hydrogen bonds with the analyzed inhibitors was identified in the molecular docking simulations, classified as hydrogen bonds strongly covalent with baricitinib (Asp197 and Leu287), chloroquine (Tyr239) and quinacrine (Tyr239), because they have bond length up to 2.5 Å and care classified as moderate and mostly electrostatic hydrogen bonds with baricitinib (Lys137), azithromycin (Leu272), hydroxychloroquine (Lys137 and Tyr237) and ruxolitinib (Lys137), by presenting a connection length up to 3.2 Å according to the parameters described in the literature [21–26]. Hydrogen bonds play an important role in macromolecular recognition, folding and stability [20]. According to classical studies [27,28], hydrogen bonds are essential in several biological processes, so the amount of these bonds can play a fundamental role in determining the molecule's interaction specificity with the pharmacological receptor.

The description of the NAK inhibitors (numb-associated kinase) effects, such as baricitinib and ruxolitinib, compared to SARS coronavirus 2, were initially described by Ref. [29]. According to the authors, these molecules would act by blocking clathrin-mediated endocytosis. Our computer tests demonstrate that the NAK inhibitors, mentioned previously, also seem to interact with the domain III of COVID-19 main protease (Fig. 2). It should be noted that, according to Ref. [6], this protein performs a primordial activity in the viral replication and transcription processes. As a comparison, ruxolitinib only showed a bond classified as moderate and mostly electrostatic hydrogen bonds in the Lys137 amino acid. According to Ref. [29,30], the selective inhibitors of JAK are potential molecules for the treatment of COVID-19. A study performed in Italian hospitals [31] indicated that the se of therapy with baricitinib enabled an improvement in the clinical characteristics and parameters of respiratory function in 12 patients suffering from pneumonia caused by COVID-19. Furthermore [32], found that the selective inhibitors of JAK can be effective in the treatment of SARS-CoV-2, by blocking viral endocytosis or reducing the presence of pro-inflammatory molecules (IFN-γ and IL-6).

In Brazil, the Ministry of Health recently introduced a new scheme for patients infected with SARS-CoV-2 in severe condition, which is based on the use of hydroxychloroquine [33]. In a study published by Ref. [34], a significant decrease in viral load was demonstrated in patients with COVID-19 after being treated with hydroxychloroquine associated with azithromycin. According to Ref. [35], hydroxychloroquine stops SARS-CoV-2 replication and invasion. On the other hand, the hydroxychloroquine docking, as well as chloroquine, did not show adequate bond energy with the enzyme. Moreover [36], presented evidence that hydroxychloroquine is ineffective for direct inhibition of the SARS-CoV-2 spike-ACE2 interaction. The weak correlation between the proposed target and the molecules does not preclude therapeutic success; however, a recent study proposed by Ref. [2] at the Shanghai Public Health Clinical Center showed that 30 patients treated with hydroxychloroquine did not show significant differences in relation to the untreated group. Besides this, chloroquine and hydroxychloroquine can lead to heart problems, associated with high doses or due to combination with antibiotics [37,38]. Another structure similar to the hydroxychloroquine analyzed was quinacrine. Our molecular docking routines demonstrated that this molecule led to the formation of three interactions with the protein target, one of the *Conventional Hydrogen Bond* type (Tyr239 (2.46 Å)). Quinacrine has long been described in the literature for human use as an oral antiprotozoal and anti-rheumatic agent. In a study performed by Ref. [39], it was suggested that quinacrine may suppress the translation directed by the Internal ribosome entry sites (IRESs) of the encephalomyocarditis virus (EMCV) and the poliovirus, indicating that this molecule may inhibit the replication of several RNA viruses. In a recent study published by Ref. [40], it was observed that quinacrine hydrochloride was able to protect 70% of mice from a lethal challenge with the Ebola virus (EBOV). From a chemical point of view, quinacrine, hydroxychloroquine, and chloroquine are lysosomotropic amines. Substances with this profile are known as endosomal acidification inhibitors, so these molecules can spread

Table 1
RMSD and affinity energy values calculated in molecular docking simulations.

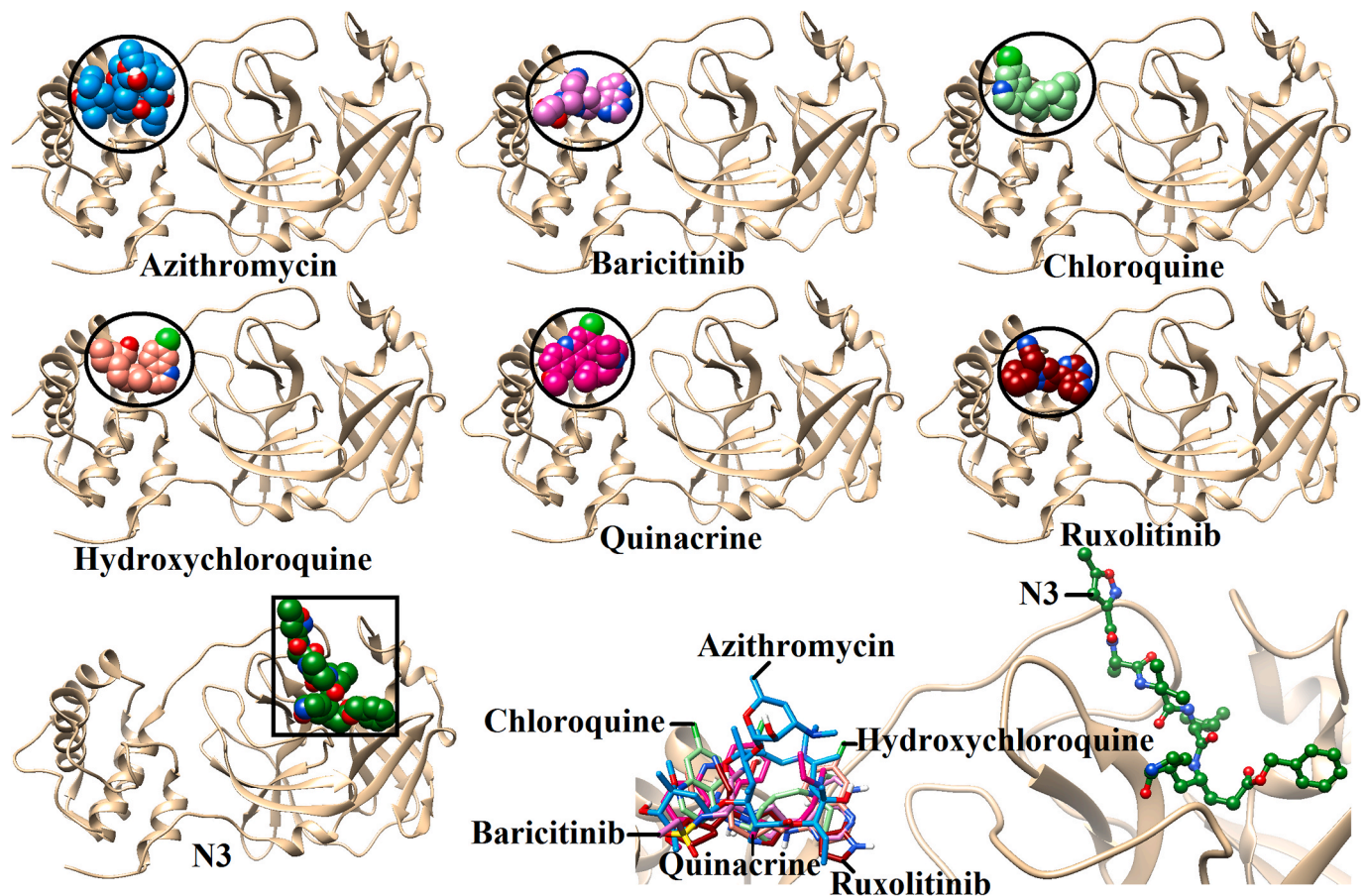| Inhibitor	Affinity (kcal/mol)	RMSD (Å)
Azithromycin	−6.3	1.060
Baricitinib	−6.3	1.432
Chloroquine	−4.7	1.687
Hydroxychloroquine	−5.0	1.978
Quinacrine	−6.0	1.728
Ruxolitinib	−6.6	1.238

Fig. 2. The ligands (Azithromycin, Baricitinib, Chloroquine, Hydroxychloroquine, Quinacrine e Ruxolitinib) binding the Mpro COVID-19 residues compared to N3.

Table 2
Distances between the M^{pro} *COVID-19* residues and the ligand.

COVID-19 (M^{pro}) residue	Azithromycin	Baricitinib	Chloroquine	Hydroxychloroquine	Quinacrine	Ruxolitinib	N3
His41	25.0 Å	23.9 Å	25.8 Å	24.9 Å	26.5 Å	24.1 Å	3.8 Å
Ser46	31.7 Å	30.8 Å	32.5 Å	31.3 Å	32.9 Å	32.1 Å	6.7 Å
Met49	27.5 Å	26.3 Å	27.9 Å	26.8 Å	28.6 Å	28.0 Å	3.9 Å
Tyr54	28.2 Å	26.5 Å	28.1 Å	27.5 Å	28.9 Å	27.6 Å	4.1 Å
Phe140	16.4 Å	15.7 Å	17.3 Å	15.8 Å	18.3 Å	14.2 Å	3.2 Å
Leu141	20.2 Å	20.1 Å	21.5 Å	20.2 Å	22.5 Å	18.8 Å	3.9 Å
Asn142	23.0 Å	22.9 Å	24.5 Å	23.2 Å	25.0 Å	21.9 Å	3.2 Å
Gly143	25.3 Å	24.9 Å	26.6 Å	25.2 Å	27.3 Å	23.8 Å	2.9 Å
Cys145	23.1 Å	22.2 Å	24.3 Å	23.0 Å	25.0 Å	21.9 Å	1.8 Å
His163	17.5 Å	16.7 Å	18.7 Å	17.3 Å	19.5 Å	16.2 Å	2.4 Å
His164	20.7 Å	19.2 Å	21.1 Å	20.0 Å	21.8 Å	19.0 Å	2.8 Å
Met165	17.3 Å	15.9 Å	18.2 Å	17.1 Å	18.6 Å	16.2 Å	3.2 Å
Glu166	15.3 Å	14.9 Å	17.0 Å	15.8 Å	17.2 Å	15.2 Å	2.8 Å
Leu167	13.2 Å	12.0 Å	13.4 Å	12.6 Å	14.2 Å	13.8 Å	4.3 Å
Pro168	13.0 Å	13.7 Å	15.2 Å	13.5 Å	15.2 Å	15.3 Å	3.5 Å
His172	12.2 Å	11.3 Å	13.4 Å	12.2 Å	14.0 Å	11.6 Å	3.7 Å
Phe185	14.0 Å	12.4 Å	13.5 Å	13.1 Å	14.3 Å	14.2 Å	7.2 Å
Asp187	23.6 Å	22.1 Å	23.5 Å	23.0 Å	24.3 Å	23.6 Å	4.0 Å
Gln189	24.7 Å	23.1 Å	24.6 Å	23.3 Å	25.3 Å	24.8 Å	2.9 Å
Thr190	20.7 Å	20.0 Å	21.3 Å	19.9 Å	22.0 Å	21.9 Å	2.8 Å
Ala191	19.4 Å	19.0 Å	20.0 Å	18.5 Å	21.0 Å	21.1 Å	3.8 Å
Gln192	15.6 Å	15.1 Å	16.2 Å	14.7 Å	16.8 Å	17.1 Å	3.7 Å

quickly through the organelles; however, in the presence of an acid pH, they become protonated and tend to accumulate in the organelles [41, 42]. In the same study performed by Ref. [34], the use of hydroxychloroquine in combination with azithromycin has been proposed. Therefore, we set out to assess whether there would be any molecular interaction between this macrolide and the aforementioned target. According to Refs. [36], azithromycin presented high affinity for

SARS-CoV-2 spike-ACE2 interaction. Some research points to the antiviral effects of the macrolides class. In a study performed by Ref. [43], the anti-rhinovial potential of azithromycin was observed, where this molecule significantly reduced the replication and release of rhinovirus. According to Ref. [44], the mechanism by which macrolides act against viruses is not well understood, but the versatility of these molecules allows their application in medical clinics. However, in a recent cohort
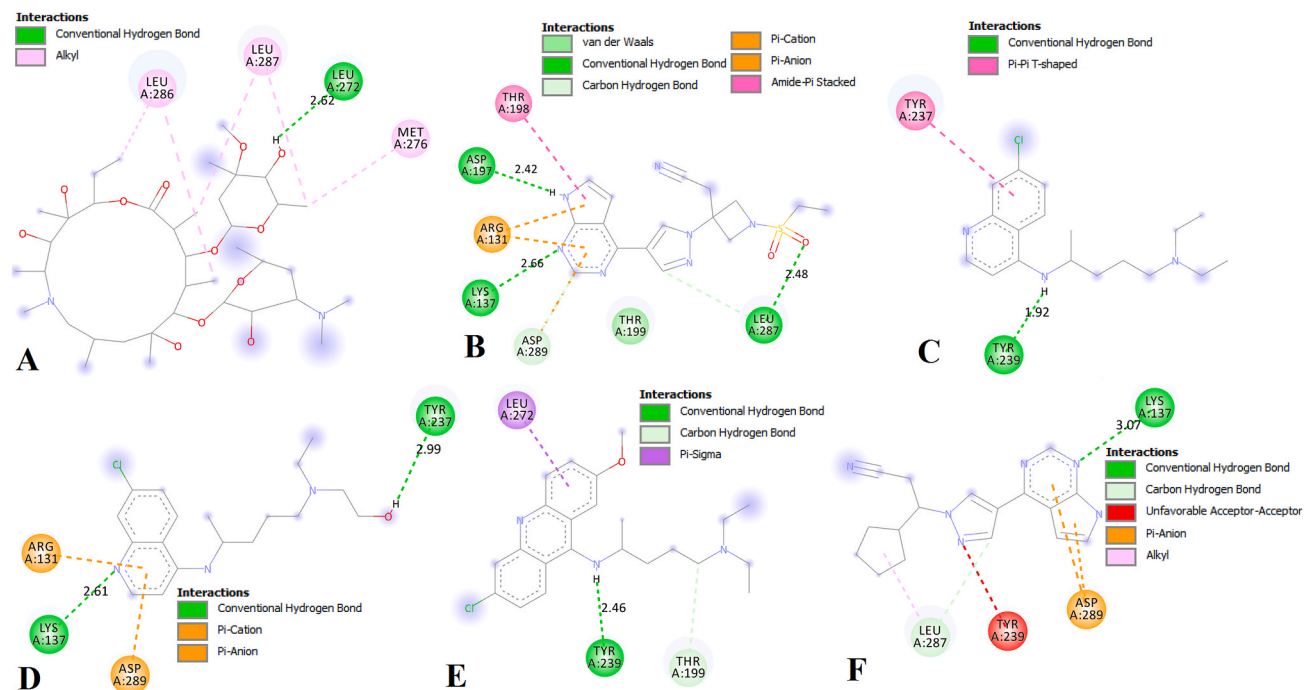
Fig. 3. Molecular interactions of the Azithromycin (A), Baricitinib (B), Chloroquine (C), Hydroxychloroquine (D), Quinacrine (E) e Ruxolitinib (F) with the Mpro COVID-19 residues.

study [45] in the New York metropolitan region indicated that treatment with hydroxychloroquine and azithromycin did not show significant differences regarding mortality rates.

4. Conclusion

The analysis of the results identified that the six inhibitors tested did not show significant distances compared to the N3 complexed in the SARS-CoV-2 main protease (M^{pro}). Although azithromycin, baricitinib, quinacrine, and ruxolitinib present bond-free energy within the standards described in the literature for affinity energy, no interactions were found with the amino acid residues from the bond site of the co-crystallized inhibitor in the enzyme, located in domains I and II. All the inhibitors analyzed showed affinity and interactions with domain III of the biological receptor. Finally, we can propose that from the results obtained from computer simulations, baricitinib, quinacrine, and azithromycin can be used alone or in combination.

CRediT authorship contribution statement

Emanuelle Machado Marinho: Conceptualization, Writing - original draft. **João Batista de Andrade Neto:** Conceptualization, Writing - original draft. **Jacilene Silva:** Conceptualization, Writing - original draft. **Cecília Rocha da Silva:** Conceptualization, Writing - original draft. **Bruno Coelho Cavalcanti:** Conceptualization, Writing - original draft. **Emmanuel Silva Marinho:** Funding acquisition, Formal analysis, Writing - original draft. **Hélio Vitoriano Nobre Júnior:** Conceptualization, Writing - original draft.

Declaration of competing interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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