

## Curcumins and its derivatives as potential inhibitors of New Coronavirus (COVID-19) main protease: an *in silico* strategy

Curcuminas e seus derivados como potenciais inibidores da principal protease do Novo Coronavírus (COVID-19): uma estratégia *in silico*

Curcuminas y sus derivados como potenciales inhibidores de la proteasa principal del Nuevo Coronavirus (COVID-19): una estrategia *in silico*

Received: 12/03/2021 | Reviewed: 12/10/2021 | Accept: 12/17/2021 | Published: 01/02/2022

**Daniela Ribeiro Alves**

ORCID: <https://orcid.org/0000-0002-0746-2211>  
Ceará State University, Brazil  
E-mail: [alves.danielaribeiro@gmail.com](mailto:alves.danielaribeiro@gmail.com)

**Matheus Nunes da Rocha**

ORCID: <https://orcid.org/0000-0002-0929-4565>  
Ceará State University, Brazil  
E-mail: [nunes.rocha@aluno.uece.br](mailto:nunes.rocha@aluno.uece.br)

**Camila Caldas Oliveira Passos**

ORCID: <https://orcid.org/0000-0002-7511-8826>  
Ceará State University, Brazil  
E-mail: [camilacaldas\\_ccop@yahoo.com.br](mailto:camilacaldas_ccop@yahoo.com.br)

**Márcia Machado Marinho**

ORCID: <https://orcid.org/0000-0002-7640-2220>  
Ceará State University, Brazil  
E-mail: [marinho.marcia@gmail.com](mailto:marinho.marcia@gmail.com)

**Emmanuel Silva Marinho**

ORCID: <https://orcid.org/0000-0002-4774-8775>  
Ceará State University, Brazil  
E-mail: [emmanuel.marinho@uece.br](mailto:emmanuel.marinho@uece.br)

**Selene Maia de Moraes**

ORCID: <https://orcid.org/0000-0002-2766-3790>  
Ceará State University, Brazil  
E-mail: [selenemaiademoraes@gmail.com](mailto:selenemaiademoraes@gmail.com)

### Abstract

Coronavirus (COVID-19) disease outbreak caused a worldwide pandemic with a powerful lethal potential and still, there is no specific treatment to it. Natural bioactive molecules like curcumins were investigated in this work aiming to block the active site of COVID-19 Main protease (Mpro), since they present several biological activities, being more suitable in terms of fewer side effects, once this disease overloads the immune system of patients. Hereby, curcumin and several derivatives were screened for their ability to react with Mpro receptors (PDB: 6LU7). N3, Azithromycin (AZT), and Baracitinib (BRT) were evaluated as positive controls and in combined therapeutics possibilities with curcumins. N3, AZT, and BRT bound to different protein receptors, and also it was observed that N3 bound in the same site as hexahydrocurcumin and curcumin glucuronide bound at the AZT's site and bisdemethoxycurcumin, curcumin, curcumin sulfate, cyclocurcumin, demethoxycurcumin, dihydrocurcumin and hexahydrocurcuminol bound at BRT's site. All molecules analyzed have high force interaction fields. Once the viral activity is mainly intracellular, these compounds also were evaluated for their hydrophobic abilities. All molecules were classified and considered capable of membrane cell invading. These results suggest that the therapeutic approach of the curcumin derivatives associated with AZT and the antiviral inhibitor N3 is promissory for future evaluation of their synergism in *in vitro* and *in vivo* tests to define their additional viability in the treatment of COVID-19.

**Keywords:** SARS-CoV-2; 3CLpro; New therapeutic tools approach; Natural products.

### Resumo

O surto da doença por coronavírus (COVID-19) causou uma pandemia mundial com poderoso potencial letal e, ainda, segue seu curso sem tratamento específico. Moléculas bioativas naturais como as curcuminas foram investigadas neste trabalho com o objetivo de bloquear o sítio ativo da protease principal (Mpro) da COVID-19, por apresentarem diversas atividades biológicas, sendo mais adequadas em termos de menos efeitos colaterais, uma vez que esta doença sobrecarrega o sistema imunológico dos pacientes. Por meio deste, a curcumina e vários derivados foram avaliados

quanto à sua capacidade de reagir com os receptores da proteína Mpro (PDB: 6LU7). N3, azitromicina (AZT) e baracitinib (BRT) foram avaliados como controles positivos e em possibilidades terapêuticas combinadas com curcuminas. N3, AZT e BRT ligaram-se a diferentes receptores de proteínas, e também foi observado que N3 ligou-se no mesmo local que a hexahidrocurcumina e o glucuronídeo de curcumina ligou-se ao local do AZT e bisdemetoxicurcumina, curcumina, sulfato de curcumina, ciclocurcumina, desmetoxicurcumina e hexahidrocurcumina no sítio do BRT. Todas as moléculas analisadas têm campos de interação de alta força. Uma vez que a atividade viral é principalmente intracelular, esses compostos também foram avaliados quanto às suas capacidades hidropáticas. Todas as moléculas foram classificadas e consideradas capazes de invadir as membranas celulares. Esses resultados sugerem que a abordagem terapêutica dos derivados da curcumina associados ao AZT e ao inibidor antiviral N3 é promissora para avaliação futura de seu sinergismo em testes *in vitro* e *in vivo* para definir sua viabilidade adicional no tratamento de COVID-19.

**Palavras-chave:** SARS-CoV-2; 3CLpro; Abordagem de novas ferramentas terapêuticas; Produtos naturais.

### Resumen

El brote de la enfermedad por coronavirus (COVID-19) ha provocado una pandemia mundial con un poderoso potencial letal y sigue sin un tratamiento específico. En este trabajo se investigaron moléculas bioactivas naturales como las curcuminas, que tienen varias actividades biológicas, con el objetivo de bloquear el sitio activo de la proteasa principal (Mpro) del COVID-19. A través de esto, se evaluó la capacidad de la curcumina y varios derivados para reaccionar con los receptores de la proteína Mpro (PDB: 6LU7). Se evaluaron N3, azitromicina (AZT) y baracitinib (BRT) como controles positivos y en combinación de posibilidades terapéuticas con curcuminas. N3, AZT y BRT se unieron a diferentes receptores de proteínas, y también se observó que N3 se unió en el mismo sitio que la hexahidrocurcumina y el glucuronídeo de curcumina se unió en el sitio AZT y bisdemetoxicurcumina, curcumina, sulfato de curcumina, ciclocurcumina, desmetoxicurcumina y hexahidrocurcumina en el sitio BRT. Todas las moléculas analizadas tienen campos de interacción de alta resistencia. Dado que la actividad viral es principalmente intracelular, estos compuestos también se evaluaron por sus capacidades hidropáticas. Todas las moléculas fueron clasificadas y consideradas capaces de invadir las membranas celulares. Estos resultados sugieren que el enfoque terapéutico de los derivados de la curcumina asociados con AZT y el inhibidor antiviral N3 es prometedor para la evaluación futura de su sinergia en pruebas *in vitro* e *in vivo* para definir su viabilidad adicional en el tratamiento de COVID-19.

**Palabras clave:** SARS-CoV-2; 3CLpro; Aproximación a nuevas herramientas terapéuticas; Productos naturales.

## 1. Introduction

The New Coronavirus (COVID-19) is a virus that belongs to the Coronaviridae family, which has a simple positive sense RNA strand, known for its high degree of contagion, which can infect a wide range of hosts, such as birds, swine and humans. COVID-19 in humans (HCOVID) has an infectious potential related to respiratory complications, ranging from the common cold to acute bronchitis and pneumonia (Fehr & Perlman, 2015; Mesel-Lemoine et al., 2012).

The disease began to manifest itself through a case of pneumonia of unknown cause in Wuhan, China, reported to the World Health Organization (WHO) of China on December 31, 2019. The coronavirus outbreak was declared an emerging state of public health and the initial milestone of the worldwide pandemic on January 30, 2020, until then with great contagious potential. According to WHO (WHO, 2020), on December 3, 2021, there were 263.563.622 confirmed cases identified in all countries, areas and territories around the world and including 5.232.562 deaths were confirmed. As a total of 7.859.585.168 vaccine doses have been administered. The symptoms of the pathology are nonspecific, such as cough, fever, and shortness of breath, however, they have a greater lethal potential (Ren et al., 2020; Rezaeetalab et al., 2020; WHO, 2021).

Main protease (Mpro), or 3C protease, responsible for viral replication, is formed by polyproteins 1A and 1AB (Hegyí & Ziebuhr, 2002; Pillaiyar et al., 2016; Wu et al., 2020; Zhou et al., 2020). Considering the importance of this protein for the vital cycle of the virus, it was used as a target in the molecular docking test, the focus of the present work, to promote new drug candidates in the treatment of COVID-19. The Mpro enzyme is a combination of two 6LU7 structures, a structural fraction used for the said molecular docking study. In previous studies, the protein was crystallized in an interaction structure with the N3 ligand, with antiviral inhibitory activity. The ligand has specific interactions with the 6LU7 protein amino acids that characterize its anchoring site, these are the interactions with CYS, where the ligand undergoes an electrophilic attack by

covalent interactions, generating a region of a strong interaction between the ligand and the receptor (Jin et al., 2020).

One of the biggest challenges in medicine is the development of antiviral resistance drugs. Therefore, it is necessary to study and develop new candidates for antiviral activity drugs extracted from natural sources, such as curcumins and their derivatives (Zandi et al., 2010). In addition to having great biological antiviral potential, curcumins are more suitable in terms of fewer side effects (Aboelhadid et al., 2019) having anti-tumor, antioxidant and anti-inflammatory activities, as well as hepatoprotective effect (Aboelhadid et al., 2019; Antiviral Potential of Curcumin, 2018; Moghadamtousi et al., 2014; Mouncea et al., 2017; Zandi et al., 2010). In combating COVID-19, they can act as inhibitors, causing direct interference in viral replication (Antiviral Potential of Curcumin, 2018). Commercially sold drugs such as azithromycin (Ulrich & Pillat, 2020) and baricitinib (Cantini et al., 2020) are adjuvant drugs with antiviral potential for the treatment of COVID-19 (Rosa & Ferreira, 2020). Together with N3, they compose the comparative ligands of the biological antiviral action of curcumins and their derivatives in this molecular docking study, to promote them as a supplementary drug in the treatment of pathology.

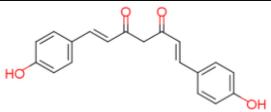
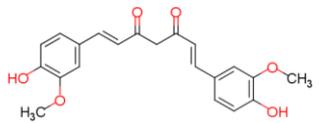
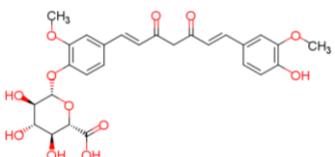
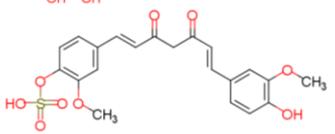
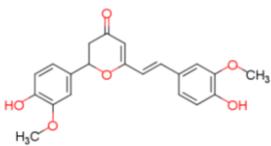
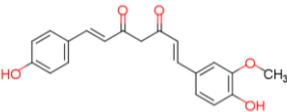
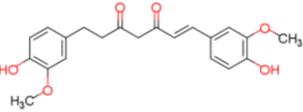
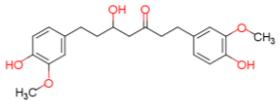
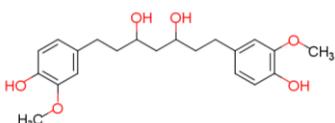
## 2. Methodology

Initially, the structure of Mpro's 6LU7 protein with the N3 ligand was reported from the RCSB protein data bank© (<https://www.rcsb.org/>) (Liu et al., 2020) and then the water molecules were removed and the file converted to .pdb protein in the UCSF Chimera® software (Pettersen et al., 2004). The two-dimensional structures of the various curcumins were obtained from the PubChem molecular repository (<https://pubchem.ncbi.nlm.nih.gov/>), then drawn and corrected in the MarvinSketch® academic software (<https://chemaxon.com/products/marvin>) (Csizmadia, 2019). Subsequently, the ligands underwent a semi-empirical geometric optimization of quantum mechanics using the parametric method 7 (PM7) using the MOPAC® software, and converted to lig.mol2. After that, the files were uploaded and submitted to the web-based tool, SwissDock (<http://www.swissdock.ch/docking#>) (Webb & Sali, 2019), Swiss Institute of Bioinformatic (SIB) server, for molecular docking simulation. Subsequently, the results received were processed in the UCSF Chimera® software, for analysis and comparison of the distances and interactions of curcumins with the interaction amino acid residues of N3, Azithromycin(AZT), and Baricitinib(BRT)(Alves et al., 2021; Rocha et al., 2021). From the distances obtained, the data were computed and plotted on the web-based tool, Morpheus (<https://software.broadinstitute.org/morpheus/>), and heatmaps were used to visualize changes in the ligand-residue interaction profiles (L-R's), being evaluated by the Pearson statistical test to detect similarity. The types of chemical interactions L-R's were analyzed and the figures were generated using the Discovery Studio ® software (Biovia et al., 2000). Then, the degrees of lipophilicity (Log P) of the ligands were analyzed to define their hydrophobic interactions, using the MLOGP method (Moriguchi et al., 1992) from the SwissADME server (<http://www.swissadme.ch/>).

## 3. Results

Among the most common interactions that comprise the 6LU7 protein catalytic sites with enzyme inhibitors, as shown in Table 1, are covalent interactions, hydrogen bonds,  $\pi$ -amide, and  $\pi$ -alkyl stacking interactions (Fokoue et al., 2020). The N3 inhibitor (control) interacts with 6LU7 by electrostatic interactions of Van der Waals through the residues of T24, T25, T26, Y54, N142, S144, D187, R188 and Q192-A, hydrogen bonds with the F140 residues, G143, H163, H164, E166, Q189 and T190, carbon-hydrogen bond with M165 and H172 residues,  $\pi$ -amide stacking with LEU141, H41, M49, M165 and L167 alkyl interactions,  $\pi$ -alkyl stacking P168, and A191 and a covalent bond with C145, forming the region of the strong interaction so that the N3 ligand binds to the protein forming the complex.

**Table 1** - Interactive and energy orders of curcumin and derivatives with 6LU7 protein.

| Compound name         | Molecular formula                                | Structural formula  | Binding affinity (kcal/mol) 6LU7 protease | Aminoacid (distance (Å))   | MLOGP <sub>alw</sub> |
|-----------------------|--|---|---|--|----------------------|
| Bisdemethoxy curcumin | C <sub>19</sub> H <sub>16</sub> O <sub>4</sub>   |    | -7.6                                      | Q19 (2.31)<br>W31 (5.56)<br>A70 (4.25)<br>G71 (2.29)<br>N95 (2.18)<br>K97 (4.33) | 2.13                 |
| Curcumin              | C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>   |    | -8.5                                      | A70 (2.56)<br>G71 (2.66)<br>K97 (4.49)<br>P122 (3.06)                            | 1.47                 |
| Curcumin glucuronide  | C <sub>27</sub> H <sub>28</sub> O <sub>12</sub>  |    | -9.0                                      | P108 (3.01)<br>E240 (1.85)<br>U246 (2.22)<br>I249 (2.43)<br>P293 (2.58)          | -1.17                |
| Curcumin sulfate      | C <sub>21</sub> H <sub>20</sub> O <sub>9</sub> S |    | -8.7                                      | G15 (2.03)<br>Q19 (3.08)<br>G71 (2.05)<br>N95 (2.48)<br>K97 (2.71)               | 0.99                 |
| Cyclocurcumin         | C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>   |   | -7.7                                      | A70 (4.29)<br>P96 (2.85)<br>K97 (4.62)<br>G120 (2.54)                            | 1.16                 |
| Demethoxy curcumin    | C <sub>20</sub> H <sub>18</sub> O <sub>5</sub>   |  | -8.7                                      | A70 (2.72)<br>K97 (2.22)   | 1.80                 |
| Dihydro curcumin      | C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>   |  | -7.9                                      | A70 (4.76)<br>G71 (2.47)<br>K97 (4.36)   | 1.55                 |
| Hexahydro curcumin    | C <sub>21</sub> H <sub>26</sub> O <sub>6</sub>   |  | -7.4                                      | L4 (1.89)<br>T25 (2.12)<br>T26 (2.73)<br>G143 (2.56)                             | 0.40                 |
| Hexahydro curcuminol  | C <sub>21</sub> H <sub>28</sub> O <sub>6</sub>   |  | -8.9                                      | G15 (2.66)<br>G19 (2.68)<br>Q69 (2.32)<br>A70 (4.31)<br>K97 (2.10)               | 1.62                 |

Source: Authors.

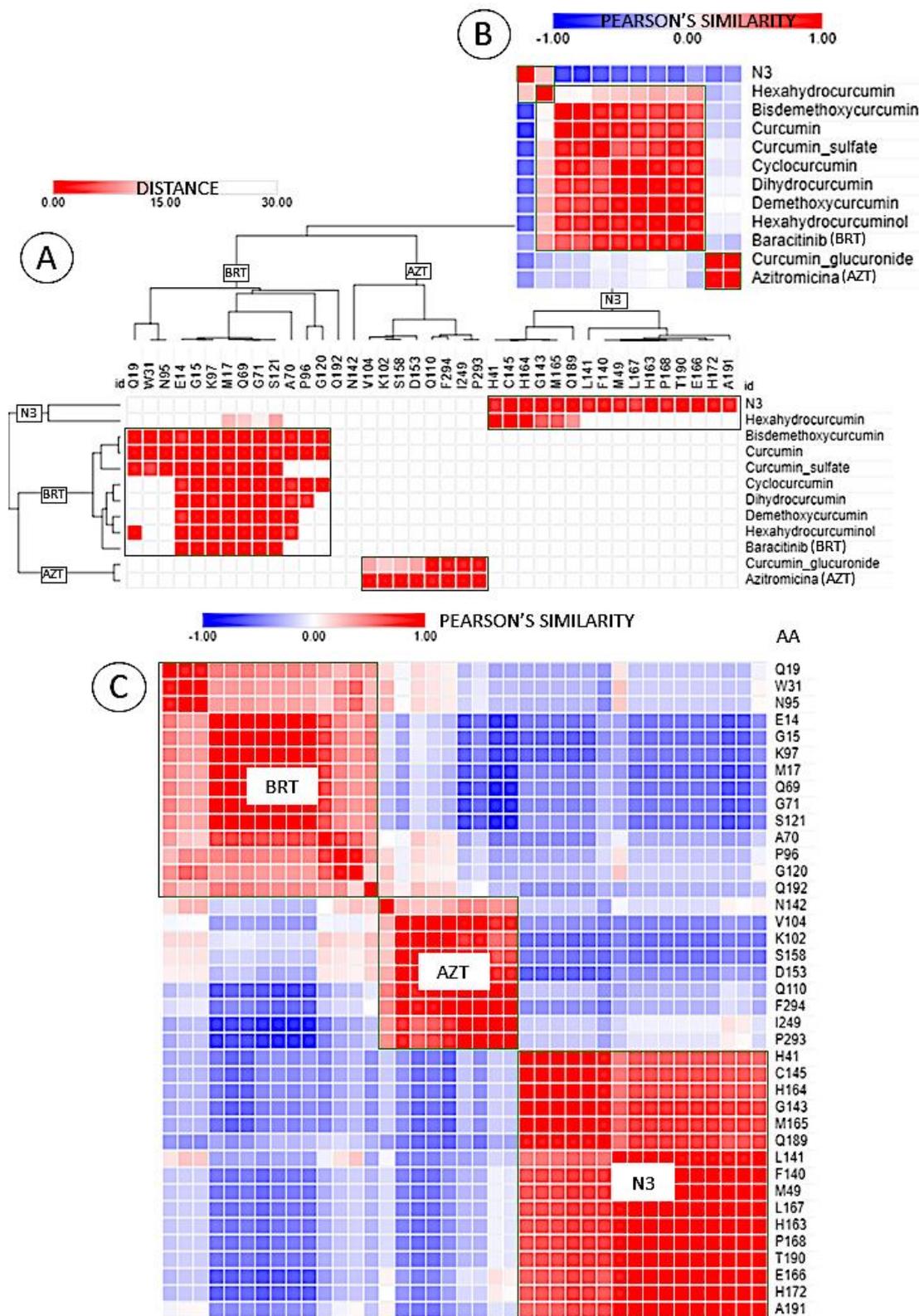
Azithromycin (AZT) is a commercially sold antiviral that was also a control binder in the present study, to evaluate its supplementary action in the treatment of COVID-19 (Rosa & Ferreira, 2020). Its interactions with the 6LU7 protein are predominantly alkylas and hydrogen bonds, which characterize a site of inhibitory activity distinct from N3. The ligand interacts with the protein through hydrogen interactions with the K102, D153 and S158 amino acid residues with a strong contribution from the hydroxyls closest to the glycoside amine group, carbon-hydrogen bonds with the Q110 and D153 residues through the donor sites of hydrogen NH and OH and alkyl and  $\pi$ -alkyl interactions with the residues of V104, I249, P293, and F294.

The baricitinib ligand (BRT), here also considered a control drug, shows only hydrogen interactions. The ligand interacts with the 6LU7 protein by hydrogen bonds with the G71 and K97 residues, with a strong contribution from the tertiary amine receptor sites of pyrimidine and the sulfate group oxygen, and carbon-hydrogen interactions with residues E14, G15, M17, Q69, and S121.

The curcumins evaluated as drug candidates in the treatment of COVID-19 were: hexahydrocurcumin, curcuminglucuronide, bisdemethoxycurcumin, curcumin, curcumin sulfate, cyclocurcumin, demethoxycurcumin, dihydrocurcumin, and hexahydrocurcuminol. After undergoing the molecular docking test, it was possible to observe that the ligands occupied the catalytic sites of the three controls (N3, Azithromycin, and Baricitinib), and this is due to the similarity of the molecular tridimensional structure among the compounds, later detailed.

The interactions of ligands and controls were mapped, as well as their energies and categories of binding were determined, thus determining the possible sites of action for all the studied molecules. It was identified that, in the results obtained in the docking and in the statistical evaluation in the Pearson similarity test (Figure 1A-C), the compounds presented grouping (clusters' formation) according to the physical-chemical and interactive similarities between themselves (L-L's), between the ligands and amino acid residues (L-R's) and between amino acids and amino acids (R-R's), as described below.

**Figure 1** - Heatmaps of the different interactions expressed between L-R's (A) Heatmaps of the different interaction forces between all ligands and their respective reactive amino acids, legitimizing three (3) active sites in the 6LU7 protein. Hierarchical clusters demonstrated. (B) Heatmap proving Pearson's similarity test reactivity between L-L's. (C) Heatmap demonstrating reactivity in Pearson's similarity test between R-R's. In the schematic, the closer to 1 (red) the interaction force will be more determinant and intense, the closer to -1 (blue) the greater the distance, and the interaction force will be negligible. Clusters highlighted by dark green squares



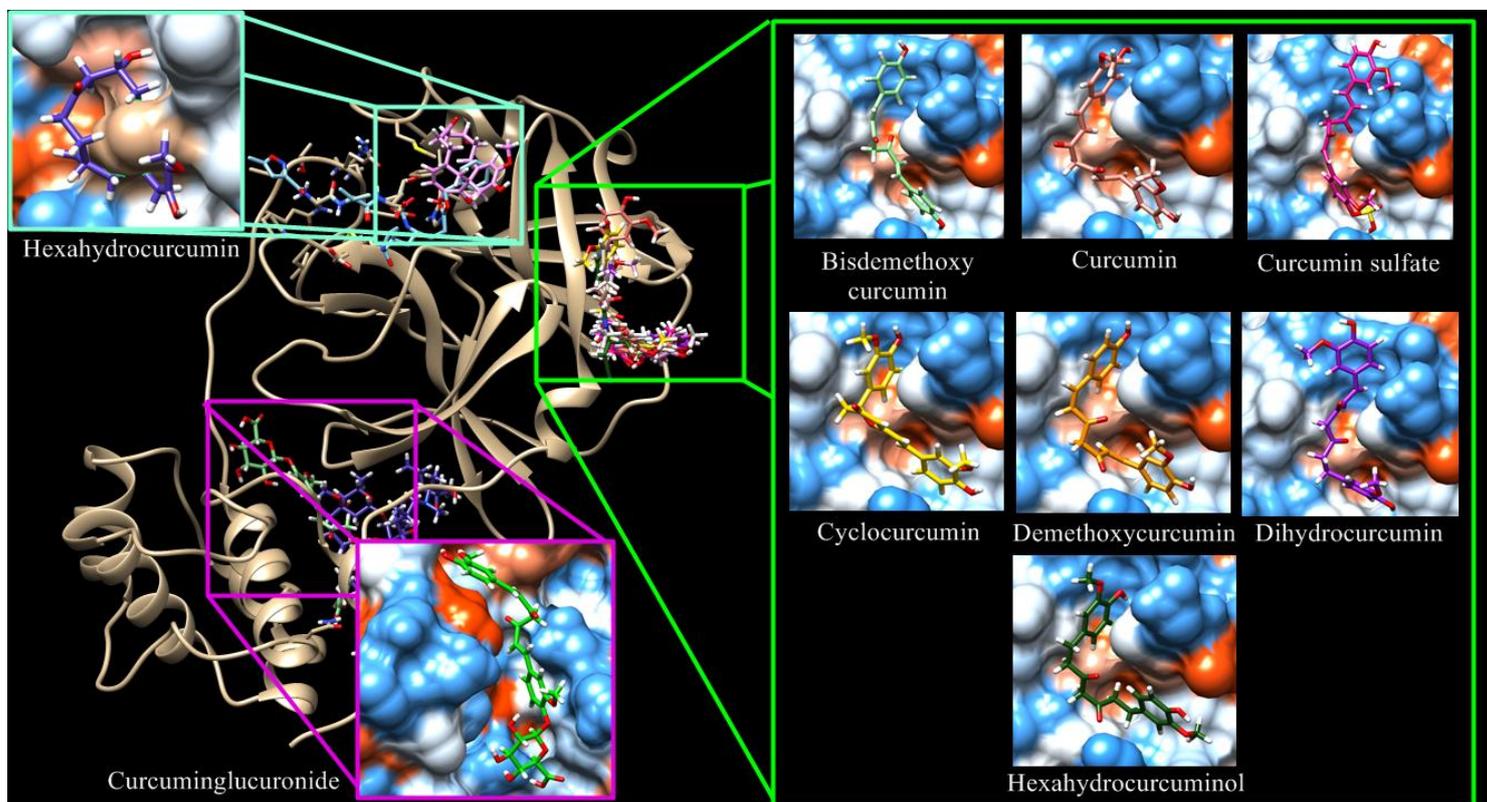
Source: Authors.

Curcumin sulfate has a sulfate group, also present in comparative ligand Baricitinib, which performs a  $\pi$ -sulfur interaction with the W31 residue of the 6LU7 protein, in addition to the interactions of conventional hydrogen bonds with the G15, G71, N95, and K97 residues, interactions carbon-hydrogen with K97 and  $\pi$ -donor interactions of hydrogen bonds with Q19.

It is worth to observe that the Curcumin and Cyclocurcumin ligands are the only compounds to interact with the protein without forming hydrogen bonds and with the same types of interaction which Curcumin interacts with the catalytic site of Baricitinib by carbon-hydrogen bonds with residues A70, G71, and P122,  $\pi$ -cation interactions with K97 and  $\pi$ -alkyl with A70. Cyclocurcumin by carbon-hydrogen bonds with P96 and G120,  $\pi$ -cation interactions with K97 and  $\pi$ -alkyl with A70. Hexahydrocurcuminol has an unfavorable donor-donor interaction with G19, hydrogen bonds with Q69 and K97, carbon-hydrogen bond with G15, and  $\pi$ -cation (K97) and  $\pi$ -alkyl (A70) interactions.

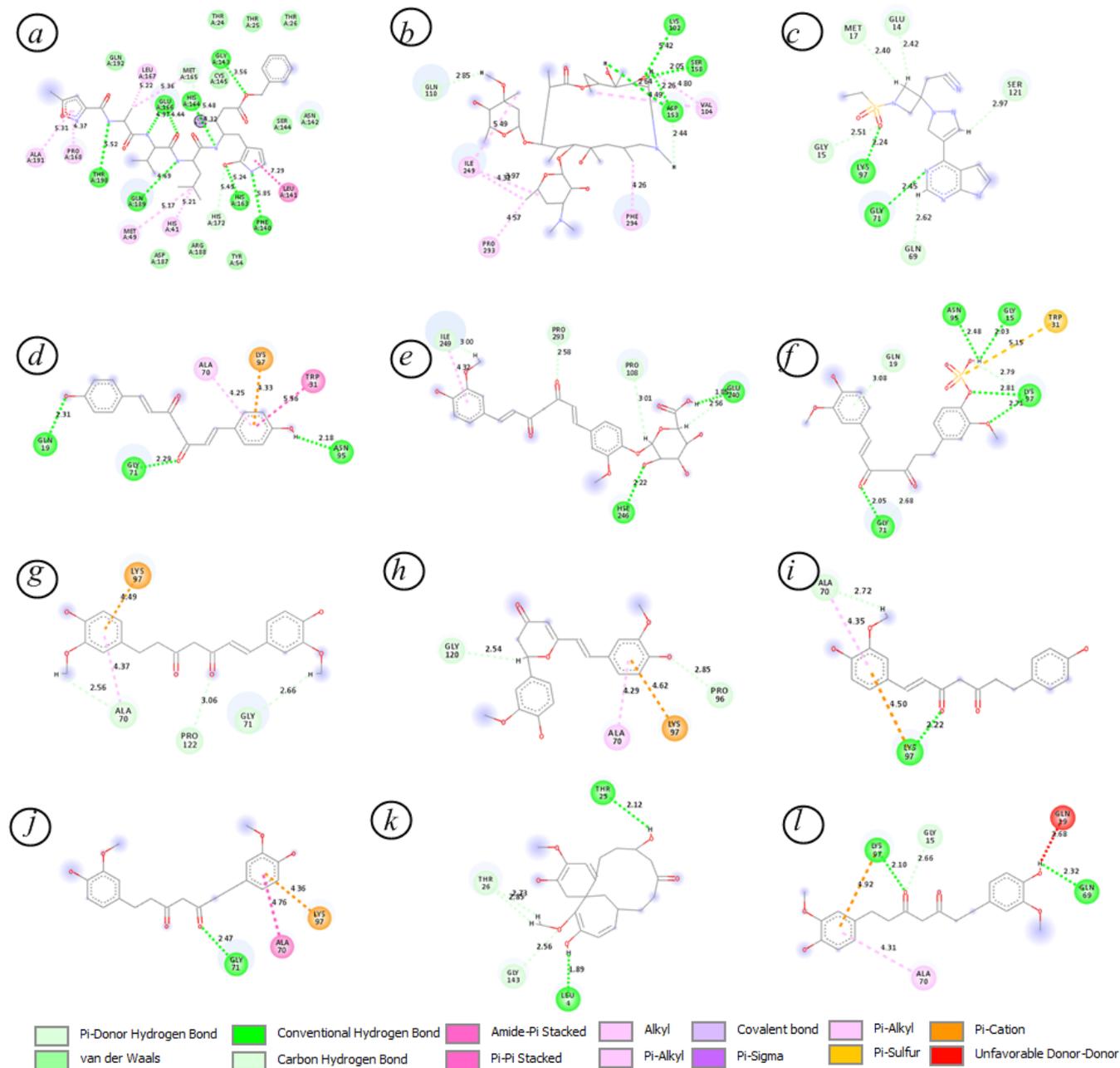
The bisdemethoxycurcumin and dihydrocurcumin ligands do not form carbon-hydrogen bonds but have  $\pi$  interactions as predominant in their bonds to the Baricitinib site. Bisdemethoxycurcumin interacts by hydrogen bonds with residues Q19, G71 and N95,  $\pi$ -cation (K97),  $\pi$ -alkyl (A70) and  $\pi$ - $\pi$  (W31) interactions, while dihydrocurcumin has a hydrogen bond (G71),  $\pi$ -cation (K97),  $\pi$ -alkyl and  $\pi$ -amide with residue A70. Demethoxycurcumin interacts with the baricitinib site for only two residues, those from A70 (carbon-hydrogen and  $\pi$ -alkyl bond) and K97 (hydrogen and  $\pi$ -cation bond). These compounds then form the largest number of drugs that are candidates for the supplementary treatment of COVID-19, performing a supplementary action with the antiviral inhibitor N3 and Azithromycin (Figures 2 and 3).

**Figure 2** - Overview of protein and spatial occupation of curcumins at N3, Azithromycin, and Baricitinib receptor sites in the approximations, a protein surface is evidenced with the evaluated ligands and their respective interaction site, where the residues are demonstrated according to their hydrophilic (blue) or hydrophobic (orange) potential.



Source: Authors.

**Figure 3** - Two-dimensional map with the regions and types of interactions of the structures a) N3; b) azytromycin; c) baricitinib; d) bisdemethoxycurcumin; e) curcumin glucuronide; f) curcumin sulfate; g) curcumin; h) cyclocurcumin; i) demethoxycurcumin; j) dihydrocurcumin; k) hexahydrocurcumin; l) hexahydrocurcuminol



Source: Authors.

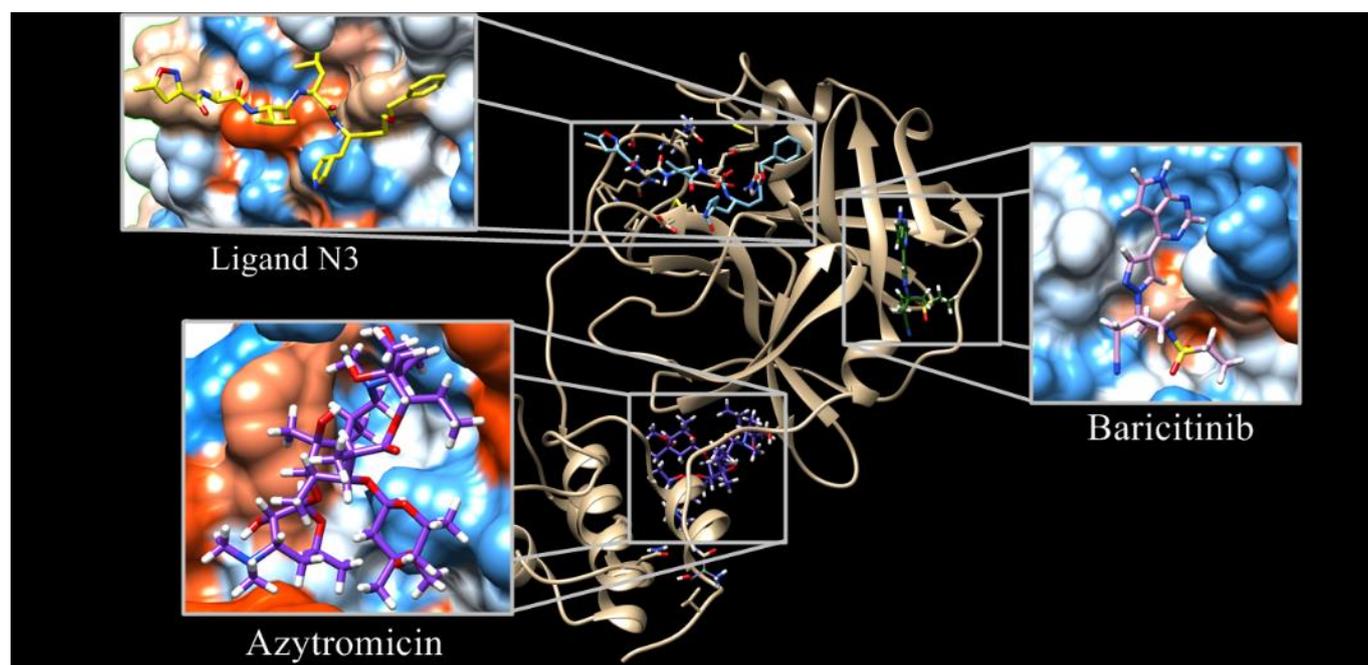
From the group of curcumin binders, it can be highlighted the interaction of Hexahydrocurcumin with a minimum energy order of  $-7.4$  kcal / mol with the receptor site of the N3 inhibitor, with a minimum distance of the residues of  $1.89\text{\AA}$  (L4) and the longest distance of  $2.56\text{\AA}$  (G143), and the curcumin ligand glucuronide as the highest energy interaction of  $-9.0$  kcal / mol, with the AZT receptor site, with a minimum residue distance of  $1.85\text{\AA}$  (E240) and a maximum of  $3.01\text{\AA}$  (P108) (Table 1).

#### 4. Discussion

For the physicochemical similarities between the ligands (Figure 1 B), a brief overlap of groups can be observed regarding the presence of the hexahydrocurcumin compound. This substance, in its composition and chemical structure, has many similarities with curcumin, the base component of the group that includes 7 compounds, such as the commercial drug BRT. Hexahydrocurcumin also has a chain arranged similarly to the N3 ligand, to have types of interactions like the control.

The hierarchical clusters there are shown in Figure 1A undoubtedly determine the poignant difference between the said active sites, corroborating with Figure 4, where several interactions will be described below. The receptor site where the N3 ligand is found indicates a region of covalent interactions, hydrogen bonds, stacking interactions  $\pi$ -amide and  $\pi$ -alkyl, with susceptibility to electrophilic attacks, while the Azithromycin and Baricitinib site have a predominance of interactions with networks  $\pi$ -alkyls,  $\sigma$ -alkyls and conventional hydrogen bonds (Figure 4) (Carey, 2011; Fokoue et al., 2020). It was observed that, both in silico evaluation and in the statistical evaluation in Pearson's similarity test (Figure 1-C), the residues were grouped according to the degree of importance of interaction and physical-chemical and interactive capacities between these and the compounds (R-L's).

**Figure 4. Receptor sites for N3, Azithromycin and Baricitinib ligands.** In the approximations, a protein surface is shown with the control ligands and their respective action sites, where the residues are demonstrated according to their hydrophilic (blue) or hydrophobic (orange) potential.



Source: Authors.

Following the aforementioned color scheme, the distinction of active residues between the controls is demonstrated and the important similarity in maintaining the reactivity of these same residues in the different ligands. The strong color marking, as well as the shortest interaction distance, made the 2 new active sites stand out. Four residues were shown to be non-specific between the N3 and BRT sites, they are M17, G71, Q69, and S121. Five other residues proved to be nonspecific between the AZT and BRT sites, they are A70, P96, G120, N142, and Q192. The interaction with residues H41, C145, H164 was observed to be statistically essential for the stability of the ligands at the N3 site. As for residues E14, G15, and K97, these proved to be statistically indispensable for the stability of the ligands to the BRT site. Interaction residues Q110, F294, I249,

and P293 were statistically considered intrinsic to the AZT site. This study highlights at least one possible catalytic triad for each site described here.

When assessing the pharmacological potential of substances, it is important to observe their molecular interactions with the active sites in the biological system. These are determined by the resultant between attractive and repulsive intermolecular forces, among them hydrophobic interactions. These interactions govern its potential for attraction or repulsion to water and consequently determine whether it is easy to cross the plasma membrane (PM), naturally composed of phospholipids. Once inside the cell, proteins (R) and the ligands (L) must be able to perform several interactions for them to meet in the intracellular environment. The compound N3 makes intimate connections with hydrophilic residues, demonstrating that it, as well as the molecules that occupy the same site, will possibly be able to cross the PM (Liu et al., 2020).

Since viral replication occurs via the intracellular route, this ability of drugs is important, as well as the protein capacity to attract ligands (Vareed et al., 2008). The active sites, described in this work, by the control molecules AZT and BRT are characterized as follows, the AZT site is found between hydrophobic and hydrophilic intermolecular forces, where the first is more prominent. These characteristics show that, like the N3 site, the binding drugs are potentially capable of crossing the PM. The BRT site has a great hydrophilic interaction, which can facilitate intracellular protein interaction and increase its virulence. Its hydrophobic core of M17 has a hydrophobic index 1.9 demonstrating that this region attracts substances capable of crossing the PM. These compounds' cell invading ability further is explained by MLOGP evaluation (Table 1)(Alves et al., 2021; Moriguchi et al., 1992; Rocha et al., 2021).

Hexahydrocurcumin was the only ligand of proximity to the active site of N3, the compound has essentially hydrogen bond interactions. The ligand interacts with the protein through conventional hydrogen bonds with the L4 and T25 residues, with a strong contribution from the hydroxyl hydrogen donor sites, and carbon-hydrogen interactions with T26 and G143. The compound is the only drug candidate, among the curcumins in this study, to demonstrate the possible synergistic effect in the treatment of COVID-19 together with Azithromycin and Baricitinib.

It is interesting to observe that the only compound that occupied the same catalytic site as Azithromycin was curcumin glucuronide, this was due to the physical-chemical and interactive similarities between the compounds. Both have a glycosidic side chain, which performs hydrogen interactions with the residues with strong contributions from the hydroxyl groups of the glucose moieties. Curcumin glucuronide interacts with the protein through conventional hydrogen bonds with E240 and U246 residues, carbon-hydrogen interactions with P108, I249, and P293 and  $\pi$ -alkyl stacking interactions with I249 and synergizes with the N3 inhibitor and Baricitinib (Figures 2 and 3).

Curcuminoid compounds have a base structure that holds aromatic rings, hydroxyls, ethers and ketones. Anti-inflammatory, anti-cancer and anti-mutagenic activities have been reported related to the presence of its ketones, as well as the double bonds present in its carbon chain. Furthermore, antioxidant activity was related to the presence of its hydroxyls. In this work, we report that the presence of hydroxyls associated with the ether and benzene groups are responsible for the potential antiviral action of the semi-synthetic curcuminoid compounds listed here, as shown in Figure 3. The cation- $\pi$  interaction occurs between the benzene group of the curcuminoid compound and amino acid nitrogen, being a non-covalent molecular interaction between the face of this electron-rich  $\pi$  quadrupole system and the adjacent monopole cation. After this interaction, the same electron-rich system interacts with another amino acid through pi-alkyl interactions, the referred amino acid, having a free valence in a saturated carbon, performing the alkyl interactions. For example, the compounds bisdemethoxycurcumin, curcumin, demethoxycurcumin and hexahydrocurcuminol (Figure 3DGIL, respectively) undergo an electrophilic attack of type  $\pi$ -cation from K97, as well as experience electrostatic attraction of the type  $\pi$ -alkyl with A70. These interactions proved to be determinant for the alteration of the three-dimensional state of the Mpro protein, potentially inactivating it.

Most of the compounds in the curcumin group in the study occupy the active site of Baricitinib, due to the similarity of their physical-chemical properties and their types of interactions, highlighting the  $\pi$ -sulfur interactions with infrequent amino acids in 6LU7, and the stacking interactions  $\pi$  with cations from the residues, called  $\pi$ -cations, in addition to hydrogen interactions with the most frequent amino acids in 6LU7 (Nelson L., David; Cox M., 2014).

Once the hydrophilic and hydrophobic regions of the protein receptor residues were known, it was possible to determine the degree of lipophilicity of the ligands by MLOGP. It can be highlighted the bisdemethoxycurcumin ligand with the highest degree of hydrophobicity and curcumin glucuronide as the ligand with the greatest hydrophilic interaction (Table 1) (Moriguchi et al., 1992; Nelson et al., 2014).

## 5. Conclusion

Considering the approximation of most ligands to the baricitinib receptor site, a therapeutic approach to the compound's bisdemethoxycurcumin, curcumin sulfate, curcumin, cyclocurcumin, demethoxycurcumin, dihydrocurcumin and hexahydrocurcuminol associated with Azithromycin and the antiviral inhibitor N3 is a promissory strategy. Then, it is suggested an evaluation of these substances in synergism with the respective drugs in in vitro and in vivo tests to define their additional viability in the treatment of COVID-19.

## Acknowledgments

We thank Ceará Foundation of Support for Scientific and Technological Development (FUNCAP) for Doctoral's degree grant (10/2018), Dean's Office of Extension from the Ceará State University (PROEX-UECE) for Initiation grants (86-87/2019). We thank the Ceará State University for the facilities to carry out this work.

## References

- Abuelhadid, S. M., El-Ashram, S., Hassan, K. M., Arafa, W. M., & Darwish, A. B. E. (2019). Hepato-protective effect of curcumin and silymarin against *Eimeria stiedae* in experimentally infected rabbits. *Livestock Science*, 221(August 2018), 33–38. <https://doi.org/10.1016/j.livsci.2019.01.011>
- Alves, D. R., da Rocha, M. N., de Sousa, D. S., Oliveira, I. C. M., Marinho, M. M., de Morais, S. M., & Marinho, E. S. (2021). Virtual Screening of Natural Curcumins and Related Compounds Against SARS-CoV-2. *Journal of Computational Biophysics and Chemistry*, 20(01), 53–70. <https://doi.org/10.1142/S2737416521500046>
- Biovia, D. S., Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., & Richmond, T. J. (2000). Dassault Systèmes BIOVIA, Discovery Studio Visualizer. *The Journal of Chemical Physics*, 17(2).
- Cantini, F., Niccoli, L., Matarrese, D., Nicastrì, E., Stobbione, P., & Goletti, D. (2020). Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. *Journal of Infection*, January. <https://doi.org/10.1016/j.jinf.2020.04.017>
- Carey, F. A. (2011). *Química orgânica*, Vol. 1 (7th ed.). Bookman Editora.
- Csizmadia, P. (2019). *MarvinSketch and MarvinView: Molecule Applets for the World Wide Web*. 1775. <https://doi.org/10.3390/ecsoc-3-01775>
- Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An overview of their replication and pathogenesis. In *Coronaviruses: Methods and Protocols* (pp. 1–23). Humana Press. [https://doi.org/10.1007/978-1-4939-2438-7\\_1](https://doi.org/10.1007/978-1-4939-2438-7_1)
- Fokoue, H. H., Pinheiro, P. S. M., Fraga, C. A. M., & Sant, C. M. R. (2020). Há algo de novo no reconhecimento molecular aplicado à química medicinal? *Quim. Nova*, 43(1), 78–89. <https://doi.org/10.21577/0100-4042.20170474>
- Hegyí, A., & Ziebuhr, J. (2002). Conservation of substrate specificities among coronavirus main proteases. *Journal of General Virology*, 83(3), 595–599. <https://doi.org/10.1099/0022-1317-83-3-595>
- Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., You, T., Liu, X. X., Yang, X., Bai, F., Liu, H., ... Yang, H. (2020). Structure-based drug design, virtual screening and high-throughput screening rapidly identify antiviral leads targeting COVID-19. *BioRxiv*, 2020.02.26.964882. <https://doi.org/10.1101/2020.02.26.964882>
- Liu, X., Zhang, B., Jin, Z., Yang, H., & Rao, Z. (2020). The crystal structure of COVID-19 main protease in complex with an inhibitor N3. *PDB Release*, 119(February), 17–20. <https://doi.org/10.2210/PDB6LU7/PDB>

- Mathew, D., & Hsu, W.-L. (2018). Antiviral potential of curcumin. *Journal of Functional Foods*, 40, 692–699. <https://doi.org/10.1016/j.jff.2017.12.017>
- Mesle-Lemoine, M., Millet, J., Vidalain, P.-O., Law, H., Vabret, A., Lorin, V., Escriou, N., Albert, M. L., Nal, B., & Tangy, F. (2012). A Human Coronavirus Responsible for the Common Cold Massively Kills Dendritic Cells but Not Monocytes. *Journal of Virology*, 86(14), 7577–7587. <https://doi.org/10.1128/jvi.00269-12>
- Moghadamtousi, S. Z., Kadir, H. A., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014). A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *BioMed Research International*, 1–13. <https://doi.org/10.1155/2014/186864>
- Moriguchi, I., Hirono, S., Liu, Q., Nakagome, I., & Matsushita, Y. (1992). Simple Method of Calculating Octanol/Water Partition Coefficient. *CHEMICAL & PHARMACEUTICAL BULLETIN*, 40(1), 127–130. <https://doi.org/10.1248/cpb.40.127>
- Mouncea, B. C., Cesaroa, T., Carraua, L., Vallet, T., & Vignuzzia, M. (2017). Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. *Antiviral Research*, 142, 148–157.
- Nelson L., David; Cox M., M. (2014). *Principios de Bioquímica de Lehninger* (7th ed.). Artmed.
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25(13), 1605–1612. <https://doi.org/10.1002/jcc.20084>
- Pillaiyar, T., Manickam, M., Namasivayam, V., Hayashi, Y., & Jung, S. H. (2016). An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: Peptidomimetics and small molecule chemotherapy. *Journal of Medicinal Chemistry*, 59(14), 6595–6628. <https://doi.org/10.1021/acs.jmedchem.5b01461>
- Ren, Z.-L., Hu, R., Wang, Z.-W., Zhang, M., Ruan, Y.-L., Wu, Z.-Y., Wu, H.-B., Hu, X.-P., Hu, Z.-P., Ren, W., Li, L.-C., Dai, F.-F., Liu, H., & Cai, X. (2020). Epidemiologic and clinical characteristics of heart transplant recipients during the 2019 coronavirus outbreak in Wuhan, China: A descriptive survey report. *The Journal of Heart and Lung Transplantation*, 39(5), 412–417. <https://doi.org/10.1016/j.healun.2020.03.008>
- Rezaeetalab, F., Mozdourian, M., Amini, M., Javidarabshahi, Z., & Akbari, F. (2020). COVID-19: A New Virus as a Potential Rapidly Spreading in the Worldwide. *Journal of Cardio-Thoracic Medicine*, 8(1). <https://doi.org/10.22038/jctm.2020.46924.1264>
- Rocha, M. N. da N. da, Alves, D. R. R., Marinho, M. M. M., Morais, S. M. D. M. De, & Marinho, E. S. S. (2021). Virtual screening of citrus flavonoid tangeretin: a promising pharmacological tool for the treatment and prevention of Zika fever and COVID-19. *Journal of Computational Biophysics and Chemistry*, S2737416521500137. <https://doi.org/10.1142/S2737416521500137>
- Rosa, G., & Ferreira, E. (2020). Therapies used in rheumatology with relevance to coronavirus disease 2019. *Clinical and Experimental Rheumatology*, 38(2), 370.
- Ulrich, H., & Pillat, M. M. (2020). CD147 as a Target for COVID-19 Treatment: Suggested Effects of Azithromycin and Stem Cell Engagement. *Stem Cell Reviews and Reports*. <https://doi.org/10.1007/s12015-020-09976-7>
- Vareed, S. K., Kakarala, M., Ruffin, M. T., Crowell, J. A., Normolle, D. P., Djuric, Z., & Brenner, D. E. (2008). Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiology and Prevention Biomarkers*, 17(6), 1411–1417. <https://doi.org/10.1158/1055-9965>
- Webb, B., & Sali, A. (2019). Docking Screens for Drug Discovery. In *Methods in Molecular Biology* (Vol. 2053). Humana. <https://doi.org/10.1007/978-1-4939-9752-7>
- World Health Organization - WHO. (2020). Coronavirus disease (COVID-19) Situation report - 104.
- World Health Organization - WHO. (2021). Coronavirus disease (COVID-19) Pandemic.
- Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., Song, Z. G., Hu, Y., Tao, Z. W., Tian, J. H., Pei, Y. Y., Yuan, M. L., Zhang, Y. L., Dai, F. H., Liu, Y., Wang, Q. M., Zheng, J. J., Xu, L., Holmes, E. C., & Zhang, Y. Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265–269. <https://doi.org/10.1038/s41586-020-2008-3>
- Zandi, K., Ramedani, E., Mohammadi, K., Tajbakhsh, S., Deilami, I., Rastian, Z., Fouladvand, M., Yousefi, F., & Farshadpour, F. (2010). Evaluation of antiviral activities of curcumin derivatives against HSV-1 in Vero cell line. *Natural Product Communications*, 5(12), 1935–1938. <https://doi.org/10.1177/1934578x1000501220>
- Zhou, P., Yang, X.-L., Lou, Shi, Z.-L. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. Di, Liu, M. Q., Chen, Y., Shen, X. R., & Shi, Z.-L. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270–273. <https://doi.org/10.1038/s41586-020-2012-7>