STUDIES OF MOLECULAR DOCKING BETWEEN LUTEOLIN 3'-(4"-ACETYLGLUCURONIDE) AND 3CL PROTEASE OF SARS-CoV-2

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ABSTRACT

The pandemic caused by SARS-CoV-2 has been the center of attention worldwide since the end of 2019. No therapeutic alternative is effective against the virus. Among the studied molecules is luteolin, a flavonoid extracted from *Lonicera japonica* Thunb. with binding affinity to the SARS-CoV-2 protease 3CL, important for viral replication. Thus, this evaluated, by molecular docking techniques, the interaction between luteolin derivatives and SARS-CoV-2 3CL. luteolin-4'-Glucoside, luteolin 6-C-glucoside 8-C-arabinoside and luteolin 3'-(4"-acetylglucuronide) derivatives were tested as 3CL ligands, all of which showed a more favorable binding energy value than that found for the standard 3N ligand (-7.8 to -8.0 kcal/mol vs -4.5 kcal/mol). The 3'- (4"-acetylglyucuronide) luteolin derivative, demonstrated for the first had the best binding value, -8.0 kcal/mol. The types of bond found between the target and the luteolin derivatives were mostly alkyl-type bonds, which may explain the more favorable values compared to the standard ligand. The results presented, combined with pharmacological activities and the low toxicity already demonstrated for luteolin, make it, together with its derivatives, a promising alternative for the treatment of COVID-19 and encourage more detailed studies.
KEYWORDS: COVID-19; Luteolin derivatives; Molecular docking.

ESTUDOS DE ANCORAGEM MOLECULAR ENTRE LUTEOLINA 3'-(4"-ACETILGLUCURONÍDEO) E 3CL PROTEASE DE SARS-CoV-2

RESUMO

A pandemia causada pela SARS-CoV-2 é o centro das atenções mundiais desde o final de 2019. Nenhuma alternativa terapêutica se mostrou eficaz contra o vírus. Entre as moléculas estudadas está a luteolina, um flavonoide extraído da *Lonicera japonica* Thunb. com afinidade de ligação à protease 3CL do SARS-CoV-2, importante para a replicação viral. Assim, este trabalho avaliou, por meio de técnicas de docking

molecular, a interação entre derivados de luteolina e 3CL do SARS-CoV-2. Derivados de luteolina-4'-glucosídeo, luteolina 6-C-glucosídeo 8-C-arabinosídeo e luteolina 3'-(4"-acetilglucuronato) foram testados como ligantes para 3CL, todos os quais mostraram um valor de energia de ligação mais favorável do que o encontrado para o ligante 3N padrão (-7,8 a -8,0 kcal/mol vs -4,7 kcal/mol). O derivado luteolina 3'-(4"-acetilglucuronato), demonstrado pela primeira vez, apresentou o melhor valor de ligação, -8,0 kcal/mol. Os tipos de ligação encontrados entre o alvo e os derivados da luteolina eram principalmente ligações do tipo alquil, o que pode explicar os valores mais favoráveis em comparação com o ligante padrão. Os resultados apresentados, aliados às atividades farmacológicas e à baixa toxicidade já demonstrada para a luteolina, tornam-na, juntamente com seus derivados, alternativas promissoras para o tratamento da COVID-19 e incentivam estudos mais detalhados.
PALAVRAS-CHAVE: COVID-19; Derivados da luteolina; *Docking* molecular.

INTRODUCTION

In December 2019, the first cases of a disease that globally imposed a new social and economic dynamics were recorded in China, Wuhan city and Hubei province (AL-TAWFIQ, 2020; LUPIA et al., 2020). COVID-19 (Corona Virus Disease) is the name of the disease and its etiologic agent is the SARS-CoV-2 virus (severe acute respiratory syndrome 2) (JI et al., 2020), previously known as Coronavirus 2019. Approximately three months later, the World Health Organization (WHO) decreed, on March 11, 2020, that Covid-19 infection was placed in the pandemic category (COVID-19 CLINICAL RESEARCH COALITION, 2020). More than a year later, in September 2021, there were already 233,136,147 confirmed cases of the disease and 4,771,408 deaths, distributed in 223 countries, areas or territories (WHO, 2021).

SARS-CoV-2 is a ribonucleic acid virus (RNA +) with an envelope and it belongs to the family of betacoronaviruses, the same as SARS-CoV (severe acute respiratory syndrome) and MERS-CoV (Middle East respiratory syndrome) (PASCARELLA *et al*., 2020). The disease caused by this, COVID-19, may include symptoms such as fever, diarrhea, pneumonia, nausea, fatigue, cough, shortness of breath, muscle pain, headache, sore throat, rhinorrhea and chest pain (GREENHALGH *et al.,* 2020, HALL JR.; JI, 2020; LO *et al*., 2020; YI *et al.,* 2020,).

Given the proportion of the pandemic, efforts are being made in the most diverse areas of knowledge, aiming at the development of new drug candidates for the treatment of COVID-19 (LIU *et al.,* 2020). Research groups around the world have dedicated themselves to studies of the newly emerged virus and, although vaccines have already been developed, understanding the interactions between different binding compounds with SARS-CoV-2 molecular targets still poses a challenge (KONG *et al.,* 2020) to be overcome, as the search for enzyme inhibitors is one of the promising therapeutic strategies for COVID-19.

In this context, an important viral molecule is the 3CL protease, an essential component for the transmission and virulence of the virus, as it cleaves and activates two proteins (pp1a and pp1b) involved in the viral replication processes (SIMABUCO *et al.,* 2021). This makes it a target for drug development (BACHA *et al.,* 2004), as inhibition of 3CL protease can reduce the severity of SARS-CoV-2 infection (HALL JR.; JI, 2020).

Among the compounds capable of inhibiting this protease, luteolin stands out, a

flavonoid found in the plant species *Lonicera japonica* Thunb (SHANG *et al.,* 2011) and which has been identified as a high-affinity ligand with the 3CL protease active sites (YU *et al.*, 2020), being therefore useful in the selection of similar new compounds that also have a strong interaction with target molecule of the new coronavirus. Thus, considering on the one hand the need for new molecular ligands, and on the other hand the scenario of global economic recession, as well as the fact that many researchers do not have infrastructure for laboratory tests, computational analysis can be used as a low-cost alternative in the early stages of discovering new antimicrobial agents (MILITE *et al.,* 2019).

The active ingredients of Honeysuckle are known to have antiviral, antioxidant and anti-inflammatory activities (SHANG et al., 2011). These are attributed especially to luteolin, which is able to inhibit production of molecules such as Tumor Necrosis Factor-α (TNF-α), interleukin 4, interleukin 6 and cyclooxygenase-2 (COX-2) (LIM, 2014).In this context, the present study aimed to evaluate, in silico, the interactions of luteolin 3'-(4"-Acetylglucuronide) with the cysteine protease 3CL, comparing them with the results obtained for luteolin and its derivatives (luteolin 6-C-Glucoside 8-C-Arabinoside and luteolin-4'-Glucoside), aiming at therapeutic indications for the treatment of COVID-19. In addition to comparing the binding stability of these compounds with the standard protease inhibitor, 3N ((N - [(5-methylisoxazol-3yl) carbonyl] alanyl-l-valyl-n ~ 1 ~ - ((1R, 2Z) - 4- (benzyloxy) -4-oxo-1 - {[(3R) -2-oxopyrrolidin-3-yl] methyl} but-2-enyl) -l-leucinamide).

MATERIAL AND METHODS

To carry out molecular docking study with the 3CL protease of COVID-19, the protein database PDB.RCSB (Protein Data Bank) (https://www.rcsb.org/) was used to search for three-dimensional resolved structure of protein. To select only chain A of the PDB ID: 6LU7 structure complex (JIN et al., 2020), the file was downloaded in .pdb format and chains that were not of interest were deleted using PyMol V 2.3.3 software (Schrödinger, LLC). The 2D structures of ligands were extracted from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (KIM et al.; 2021) and ZINC (http://zinc.docking.org/substances/home/) (STERLING; IRWIN, 2015) databases and were then saved in 3D in Marvin Sketch v. 15.7.13.0 (ChemAxon, Hungria) and transformed into .pdbqt file by AutodockTools V1.5.6 program (SANNER, 1999).

The following substances were used as ligands: the 3N compound, considered a standard ligand for this protein, was recovered from pdb ID: 6LU7 structure complex, with resolution 2,16 Å; luteolin (PUBCHEM ID: 5280445), a flavonoid that has already been well used in research against COVID-19; and 3 derivatives: luteolin-4'-Glucoside (PUBCHEM ID: 12304737); luteolin 6-C-Glucoside 8-C-Arabinoside (ZINC4098553), and luteolin 3'-(4'-Acetylglucuronide) (ZINC14757131).

The AutodockTools V1.5.6 program (SANNER, 1999) was used in the preparation of ligand and receptor molecules, and the files were saved in *.pdbqt* format for use in anchorage calculations. For the preparation of the configuration file we used coordinates of grid box (x: -28.059, y: 9.486 and z: 61,528) of the work of Yu et al. (2020).

The AutoDock Vina (TROTT; OLSON, 2010) was used to calculate molecular docking and obtain the values of free energy (kcal/mol) for each conformation of each ligand with the 3CL protease. Calculations for the docking between 3CL and luteolin and derivates were performed using AutoDock Vina software (TROTT; OLSON, 2010) considering 9 different docking poses for each ligand.

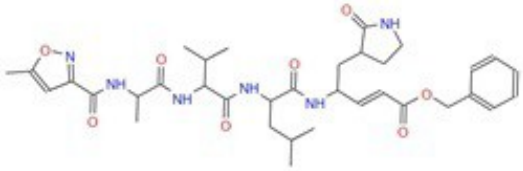
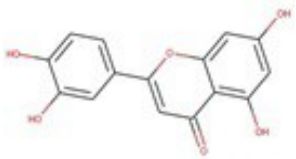
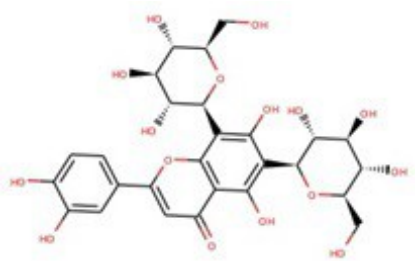
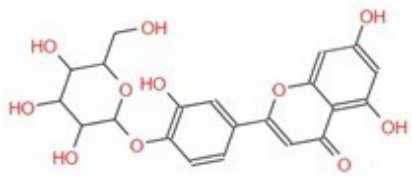
Lastly, the PyMol V 2.3.3 software (Schrödinger, LLC) was used to verify the ideal complex considering all stereochemical aspects previously evaluated, as well as the results of free energy. Discovery Studio v20.1.0.19295 (BIOVIA, 2019) was used to analyze the protein-ligand coupling, using the 2D and 3D diagram, which identifies the types of interactions that happen between the amino acids of protein with ligand.

The use of data from the genome of the species *Lonicera japonica* Thunb. was registered in the National System for the Management of Genetic Heritage and Knowledge, which generated the following code: A7CB8DD.

RESULTS

The values of binding free energy obtained for both luteolin and its derivatives against the 3CL ligand, which ranged from -7.8 to -8.0 kcal/mol, were more favorable than that shown by standard 3N ligand, from - 4.5 kcal/mol (Table 1). The root mean square deviation (RMSD) values ub (upper) and lb (lower) are for atoms with similar characteristics of one position of the ligand compared to another subsequent position of the same (LI; SHAH, 2017) (Table 1).

TABLE 1. 2D structure of ligands and interaction of luteolin and its derivatives with cysteine protease 3CL.

Ligands	2D structure of ligands	Affinity (kcal/mol)	RMSD l.b/u.b
3N		-4.5	0.000/0.000
Luteolin		-7.8	0.000/0.000
Luteolin 6-C-Glucoside 8-C-Arabinoside		-7.8	0.000/0.000
Luteolin-4'-Glucoside		-7.9	0.000/0.000

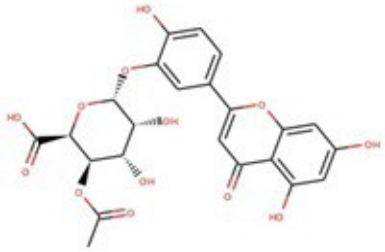
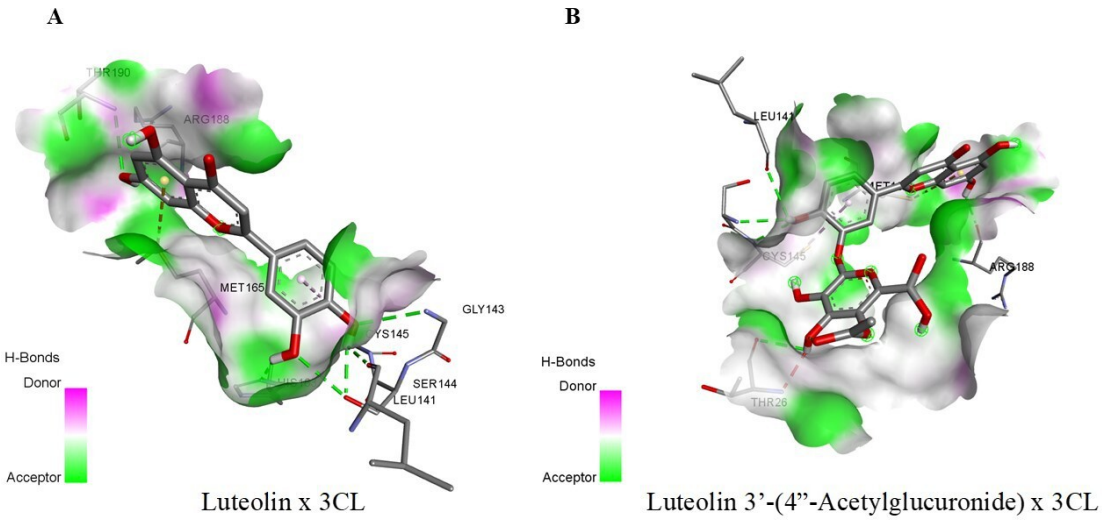
Luteolin 3'-(4"-Acetylglucuronide)		-8.0	0.000/0.000

Figure 1 shows the 3D anchoring structures between ligands and the target molecule 3CL, and Figure 2 shows patterns of molecular interactions and their respective residues in 2D.

The 3D images (Figure 1) reinforce results in Table 1, when comparing luteolin with luteolin 3'-(4"-Acetylglucuronide), as there is a greater amount of carbonyls in aromatic and carboxylic ketone forms (Figure 1, in regions on periphery of molecule, in red) in derivative, conferring in context a higher charge density of electrons in donor oxygen.

FIGURE 1: 3D structures of bonds between luteolin (A), luteolin 3'-(4"-Acetylglucuronide) (B) and the target.



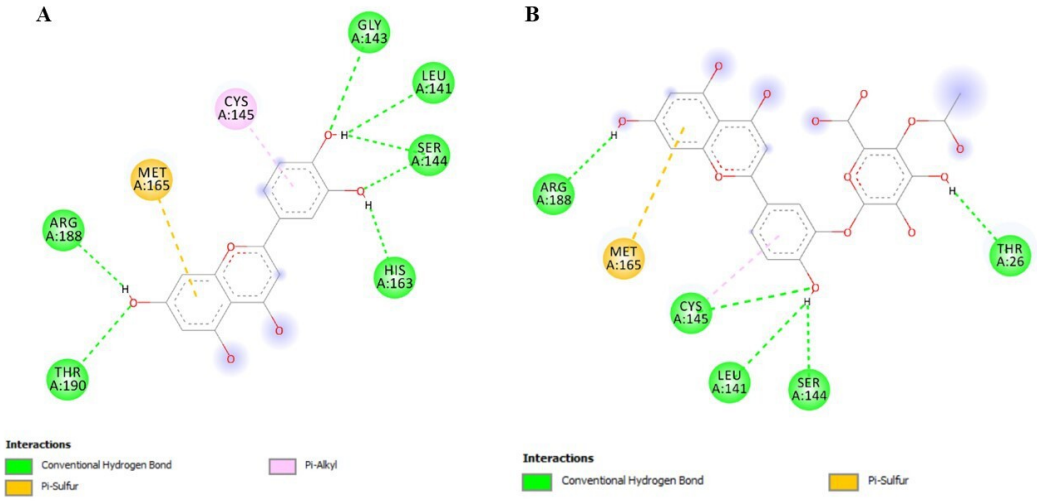
Luteolin x 3CL

Luteolin 3'-(4"-Acetylglucuronide) x 3CL

When evaluating interactions shown by luteolin against the target, we found both hydrogen bonds, in residues 141-LEU, 143-GLY, 144-SER, 163-HIS, 188-ARG and 190-THR, and bonds with alkyl groups by sulfur (145-CYS and 165-MET) (Figure 2 A).

Among all of our results, the one that showed the greatest inhibitory and unprecedented potential was obtained by molecular docking between ligand luteolin 3'-(4"-Acetylglucuronide) and the target enzyme of this study. Most of the interactions presented were stronger (26-THR, 141-LEU, 145-CYS, 188-ARG with hydrogen bonds; 165-MET with pi bond) (Figure 2 B).

FIGURE 2: Molecular interactions between the 3CL target and luteolin (A) and luteolin 3'-(4"-Acetylglucuronide) (B).



Luteolin x 3CL

Luteolin 3'-(4"-Acetylglucuronide) x 3CL

It is worth mentioning that all results presented refer to best position of ligand with target enzyme.

DISCUSSION

Situations evidenced in the course of the pandemic, such as indiscriminate use of drugs with unproven efficiency, as well as occurrence of subsequent side effects (*e. g.* cardiac with chloroquine; CHATRE et al., 2018), stimulate the search for a natural alternative ligand that has a chemotherapeutic target intrinsic to SARS-CoV-2, but it is different from the surface ones, such as Spike protein, which has been widely discussed (HALL JR.; JI, 2020; SHEHROZ *et al.*, 2020). Thus, based on these criteria and studies with a similar approach, our group chose the 3CL protein as a potential target in search for therapeutic options against SARS-CoV-2.

According to Kollman and Allen (1972) one of principles of intensity of hydrogen bonds depends on a greater charge density of electrons, thus giving greater intensity to hydrogen bonds, as well as number of carbonyls (oxygens), in context this work, offering more receptor regions on enzyme with a stronger anchorage between a 3'- luteolin (4'-acetylglucuronide) and target protein, when compared to luteolin.

Regarding the results of the work by Yu *et al*. (2020), the luteolin ligand interacted with the 3CL protease through five hydrogen bonds in GLN-189, LEU-4, ASN-142 and THR-26, respectively, as well as hydrophobic interactions with MET-49 and VAL-3. In our work, interactions/bindings of 3CL protease with luteolin were found as: conventional hydrogen bonds in amino acids LEU-141, GLY-143, HIS-163, 188-ARG, 190-THR; Pi-alkyl with 145-CYS; finally, 165-MET. These results explain the better affinity of luteolin in our results (-7.8 kcal/mol), when compared to Yu et al. (2020) (-5.37 kcal/mol), because, in addition to the number of connections being larger, the types of connections are more consistent.

Yu *et al.* (2020) performed molecular docking with luteolin against the same target

chosen in our work and also used the 3N molecule as a control ligand. In line with results found in our study, the values of free energy in bonds between 3CL and 3N control were less favorable (-3.63 kcal/mol vs -4.5 kcal/mol) than value found when the ligand used was luteolin (-5.37 kcal/mol vs -7.8 kcal/mol). Yu *et al.* (2020) ligand 3N showed, mostly, hydrophobic interactions. This may explain lower value of bond-free energy found in our assessment.

In comparison, in molecular docking with luteolin evaluations carried out by Yu *et al.* (2020) were only hydrogen bonds and hydrophobic interactions. Although there were the same amounts of amino acids, the types of interactions were more consistent in our results. Number and types of interactions reflect in affinity of ligand to its protein active/catalytic site, especially the latter criterion (TAO *et al.*, 2020).

Based on results obtained with luteolin, we tested molecular docking with its derivatives: luteolin-4'-Glucoside, luteolin 6-C-Glucoside 8-C-Arabinoside and luteolin 3'-(4"-Acetylglucuronide), as ligands. Such derivatives showed promising results, especially for the last mentioned (-7.9 kcal/mol, -7.8 kcal/mol and -8.0 kcal/mol, respectively). Data obtained here were compared with presented by Yu *et al.* (2020) for this target protease.

Other results of interactions and binding free energy found by Gheware *et al.* (2020) of luteolin-6-glycoside-8-arabnoside with the protease in question (pi and hydrogen bonds, -8.2 kcal/mol), when compared to those found by our group, show the initial premise that types of bonds interfere in the result of free energy of binding. However, in a more recent study, from the same group, this compound also makes cation-π interaction with residue (HIS 41) of 3CL with higher affinity -11.59 kcal/mol (GHEWARE *et al.*, 2021).

Molecular docking performed by Mishra et al. (2020) for luteolin-4-glycoside showed a binding free energy value very close (-7.87 kcal/mol) to that found in this work (-7.9 kcal/mol) both with the 3CL protease, but with a pattern of amino acid residues and distinct interactions.

While Mishra *et al.* (2020) reported hydrophobic and hydrophilic interactions (e.g., 280-GLU hydrogen bonds), we found most hydrophilic interactions for same interaction. These distinct patterns of interactions may have contributed to this small difference in values of binding free energy.

The ethanolic extract of leaf of *Lonicera japonica* does not have apparent toxicity in organs of rats at high concentrations as 5,000 mg/kg (THANABHORN et al., 2006), allied to this, ADMET analysis confirm this characteristic to luteolin (SHAWAN *et al.,* 2021).

CONCLUSION

From results found, it was observed that luteolin 3'- (4" - Acetylglucuronide) stood out when compared to other ligands, which presented free energy of binding with target protease of -8.0 kcal/mol. This value is lower than those found for commercialized drugs, such as chloroquine, remdevisir and ribavirin (all greater than -6.0 kcal/mol), even when compared to 3N control. Thus, this work suggests another possible ligand to be evaluated in silico (ADMET), in vitro and in vivo studies aimed at combating SARS-CoV-2 and emerging viral variants.

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