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Molecular docking identification for the efficacy of natural limonoids against COVID-19 virus main protease



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ABSTRACT

COVID-19 pandemic is the biggest public health problem of the century so far. The main protease (Mpro) is one of the main enzymes studied as a pharmacological target. In this context, the present work aimed to perform a virtual screening of possible inhibitors against the enzyme Mpro, having limonoids as the main object of research as supposed inhibitors. Molecular docking simulations indicated that limonoids have an affinity to complex with M-pro. However, Limonine and Nimoliciol showed nonspecific and low affinity interactions. In conclusion, Limonoids are substances of natural origin that can be used in the study of new pharmacological tools designed to combat and understand COVID-19.

1. Introduction

The virus that causes severe acute respiratory syndrome (SARS-CoV-2) is the etiologic agent of Coronavirus Disease (COVID-19), which took on the pandemic proportion, standing out as an important public health problem, which if not properly treated, it can result in the death of patients. Spread of this disease first occurred in Wuhan City, China, a state of threat to public health was declared, and the strict start of social isolation [1]–[4]. The virus causes a respiratory syndrome through the infection of respiratory cells and then viral replication occurs due to the translation of capsid proteins and enzymes, followed by post-translational modifications, highlighting the role of the main protease (Mpro), which becomes active after an autocleaving process [5]. Several studies of theoretical and practical character reveal a region of enzymatic inhibition, signaled by the interaction of the theoretical inhibitor N3, helping in the screening process of other compounds that inhibit enzymatic activity [6,7].

In this context, the prospecting of molecules of natural origin has been encouraged. In the present work, limonoids, structures of oxidized tetranortriterpenes originated through the oxidation of four carbons, of

their lateral structure were studied and, thus, it provides the formation of a furan ring, having the precursor structure of Euphadienol. Several limonoids have described antiviral activity [8], therefore, the present study aims to study the antiviral potential of limonoids Calodendrolide, Desacetylespathelina, 6 α -O-acetyl-7-deacetylnimocinol, Harrisonin, Limonine, Nimoliciol, Pedoninaa and Euphadienol in a screening *in silico* on inhibition of SARS-CoV-2 Mpro protease. The use of these compounds is due to their antiviral potential and, therefore, they were chosen to be tested against the SARS-CoV-2 Mpro enzyme [7].

2. Methodology

2.1. Obtaining molecular structures

The structures of Euphadienol (EPD), limonine (LMN), 6 α -O-acetyl-7-desacetylespathelin (DSP), calodendrolide (CLD), Pedonin (PDN), harrisonin (HSN), Nimoliciol (NCL) and 6 α -O-acetyl-7-deacetylnimocinol (DNM) studied by Garcez et al., 2013 [9] were selected for evaluation of their interaction with Mpro. The structures were designed with MarvinSketch software (<https://chemaxon.com/products/marvin>).

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Additionally, some drugs clinically used in the treatment of COVID-19 were tested. The following structures were imported from the virtual repository PubChem© (<https://pubchem.ncbi.nlm.nih.gov/>): Anakinra (ANK) (PubChem CID: 139595263), Azithromycin (AZT) (PubChem CID: 447043), Baricitinib (BRT) (PubChem CID: 44205240) and Remdesivir (RDS) (PubChem CID: 121304016). Moreover, N3, which is a theoretical Mpro inhibitor, was tested for comparison.

2.2. Structural optimization

Geometry optimization is a technique that aims to find a set of coordinates that minimize the potential energy of the system under study [10]. The basic procedure is to move over the potential surface in the direction in which the energy decreases so that the system is brought to a near local minimum energy. Minimization makes use of only a small portion of the configuration space. However, by adjusting atomic positions, it relaxes distortions in chemical bonds, bond angles, bond lengths, and torsion angles. The ligand structures were optimized using the classic MMFF94 force field formalism (Merck Molecular Force Field 94) [11]. The optimization calculations were performed on the Avogadro Avogadro® code [12], configured to perform force field simulations MMFF94, using the Steepest Descent algorithm, 500 numbers steps, and 10e-7 convergence parameter.

2.3. Molecular docking analysis

The three-dimensional structure of the main protease (Mpro) of SARS-CoV-2 was obtained through Protein Data Bank repository (<http://www.rcsb.org/>) (PDB ID: 6LU7), having a resolution equivalent to 2.16 Å [13]. The delimitation of gridbox parameters is essential because it directs the simulation area, in this work the gridbox was defined to encompass the entire protein structure (Fig. 1). The parameters used were: 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. using code AutodockTools version 1.5.6 [14], polar hydrogens from Mpro and ions were added [15–17].

Molecular docking simulations were performed using Vina software version 1.1.2. For each analysis, 100 cycles of 10 independent simulations were performed using the Lamarckian algorithm [18]. Docking scores are used (as a selection criterion, and this value must be equal to or less than -6.0 kcal/mol and RMSD (Root Mean Square Deviation) less than 2.0 Å were used as criteria [19].

Based on observations of the interactions between the molecules and

the enzyme, the hydrogen bonds were plotted and classified according to previous studies that group interactions with distances between 2.5 and 3.1 Å as strong, from 3.1 to 3.55 Å as average and > 3.55 Å as weak [20]. To analyze the results and plot the maps, the UCSF Chimera 1.8 (<http://www.cgl.ucsf.edu/chimera/>), LigPlot+ and the Discovery visualizer were used.

3. Results

Previously, baicalein and quercetin presented potential interactions with ACE2 receptor, by molecular docking, which can support its use in patients with severe COVID-19 [21]. In this way, molecular docking studies have been used in screening of new substances that interact with SARS-CoV-2 proteins, as the Mpro, RdRp and PLpro [15,16,22] [–] [25]. For example, andrographolide from *Andrographis paniculate* presented a potential of Mpro inhibition, and other study showed that some flavonoids and indole-chalcones might impair SARS-CoV-2 infection and replication through enzymatic inhibition [26,27]. Otherwise, molecular docking studies are main used in screening and development of new anti-SARS-CoV-2 drugs, based on pharmacophore interactions and drugs repurposing, reinforcing the versatility of this method [28,29].

Through molecular docking simulations, the interaction positions of the selected limonoids with Mpro were obtained, which are illustrated in Fig. 2. It was observed that Euphadienol (EPD), 6 α -O-acetyl-7-deacetylspathelin (DSP), Calodendrolide (CLD), Pedonin (PDN), Harrisonin (HSN) and 6 α -O-acetyl-7-deacetylnimocinol (DNM) bonded in a common region, which is the same location for Anakinra (ANK), Azithromycin (AZT), Baricitinib (BRT) and Remdesivir (RDS) (site A). In contrast, Limonin (LMN) and Nimolicinol (NCL) showed interactions in a different region (Site B). N3 docked in another separate site, which was not common for any other one of the molecules used in this work. All simulations were performed based on the energy and specific RMSD values of each worked molecule and with the observation of their interactions with the enzyme.

Therefore, in order to analyze the intensity and affinity interactions for each ligand, interaction energy and RMSD values were obtained (Table 1). It was observed that the reference ligands presented good binding energy, between -6.2 and -6.9 kcal/mol. The limonoids studied on this work performed even better, with satisfactory interaction energy and, in some cases, higher values, such as those obtained for DSP (-7.5 kcal/mol), LMN (-7.4 kcal/mol) and PDN (-7.6 kcal/mol). As validation criterion, RMSD <2.0 Å was adopted, which was observed for all ligands.

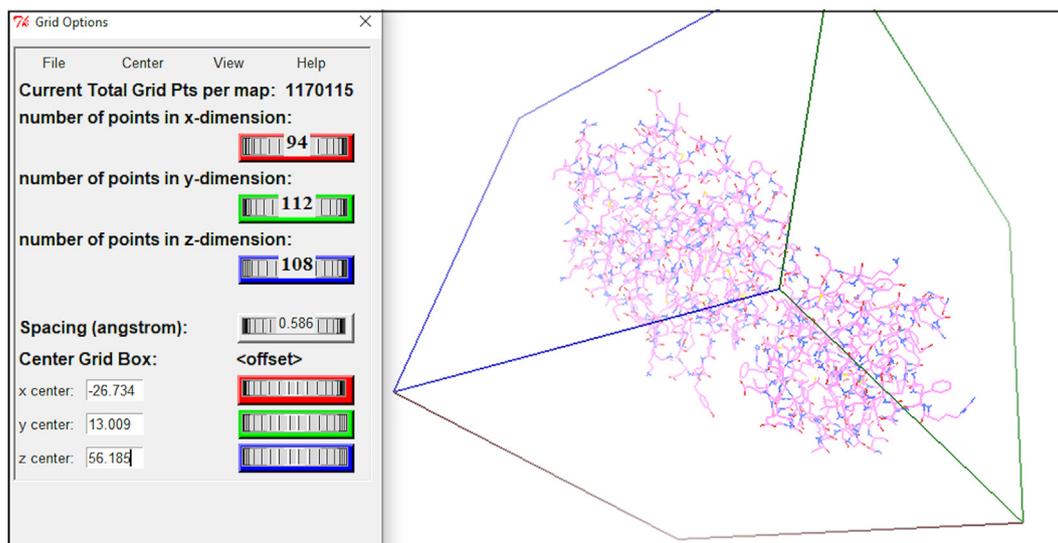


Fig. 1. Grid box parameters used to perform a simulation.

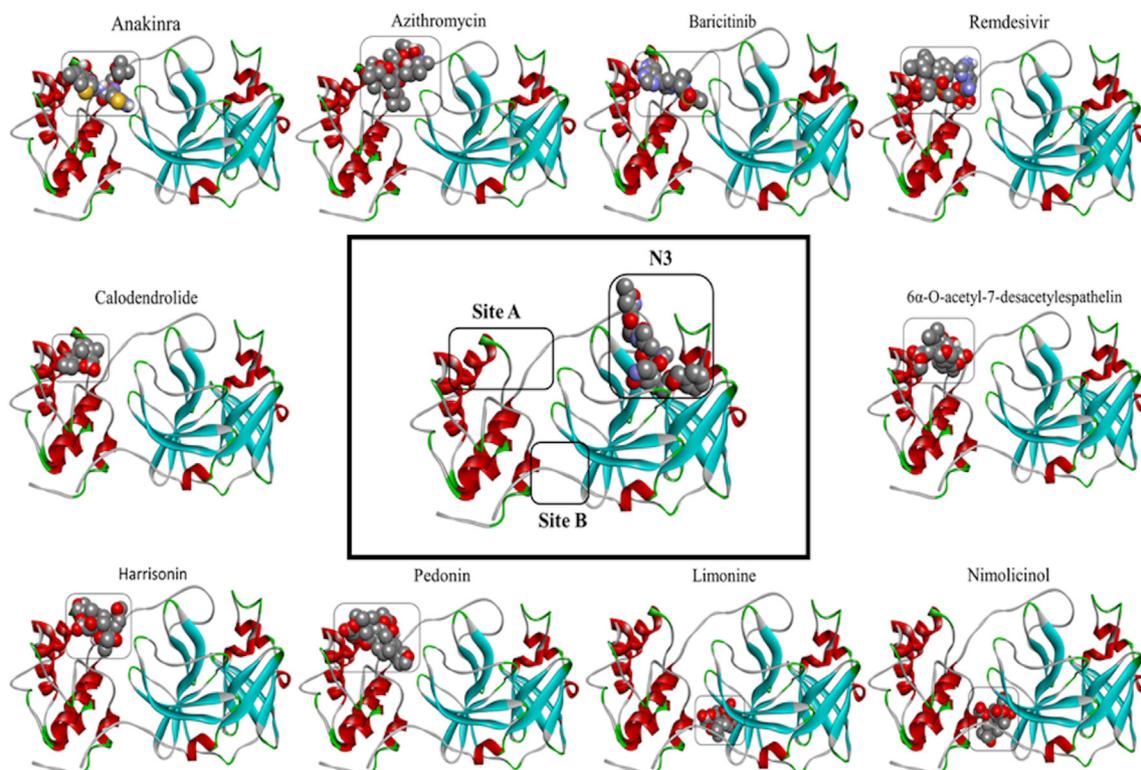


Fig. 2. Interaction positions of limonoids, drugs used as comparative binders and N3 inhibitor with Mpro in the molecular docking simulation.

Table 1

Interaction energy and RMSD of limonoids and reference ligands in molecular docking assay with SARS-CoV-2 Mpro.

Ligands	Vina docking score(kcal/mol)	RMSD
Limonooids	-6.0	1.438 Å
CLD		
DSP	-6.2	1.904 Å
DNM	-7.5	1.551 Å
HSN	-6.2	1.831 Å
LMN	-7.4	1.835 Å
NCL	-6.8	1.731 Å
PDN	-7.6	1.683 Å
EPD	-7.0	1.504 Å
Reference ligands	-6.2	1.422 Å
ANK		
AZT	-6.9	1.646 Å
BRT	-6.8	1.332 Å
RDS	-6.8	1.728 Å

CLD – Calodendrolide; DSP - 6 α -O-acetyl-7-desacetylespathelin; DNM - 6 α -O-acetyl-7-deacetylnimocinol; HSN – Harrisonin; LMN – Limonin; NCL – Nimolicinol; PDN – Pedonin; EPD – Euphadienol.

Aiming to analyze and describe the intrinsic characteristics of interactions with Mpro, hydrogen bonds were specified. In Fig. 3 it is possible to observe the interactions of hydrogen with some limonoids. ANK, AZT, BRT and RDS showed similarities in their interactions with Mpro. It is noteworthy that ANK, AZT and BRT interacted directly with Thr199 (A), similarly to CLD, NCL and DNM.

Table 2 shows the interaction distances between the compounds studied in the present work and the amino acid residues of the enzyme Mpro. It was observed that, among the reference ligands, most of the connections have classifications between strong and moderate (<3.1 Å). Furthermore, AZT was the drug that had the highest frequency of weak interactions, with a total of four, followed by BRT, with three and ANK with two. RDS showed two strong interactions (Arg131 and Asp289),

despite the weak interaction with Thr199.

Comparatively, among the limonoids, LMN and NCL were far from the protein residues, the smallest being, for example, the interaction of Nimolicinol with Asp289 (11.13 Å). These data corroborate the hypothesis that the interaction of these two compounds with enzyme sites different from the other limonoids and reference ligands may occur through a non-specific and low intensity interaction. In contrast, PDN presented the best affinity with Mpro, with most of its connections of moderate intensity. However DSP, DNM and HSN interacted with Thr199 with a strong intensity, similar to the reference ligands.

4. Discussion

In the present work, it was demonstrated that the most of the limonoids studied presented high-energy theoretical interactions with Mpro at similar sites to clinically used drugs, based on the obtainment of computational data, to verify the positions of each studied limoid, but these analyzes point out differences when compared to the theoretical inhibitor N3. The reference ligands showed hydrogen bonds of strong to moderate intensity with Thr199, a pattern that was repeated among the limonoids. In contrast, limonine and nimolicinol showed nonspecific and low affinity interactions.

These results are important because the viral genetic material encodes enzymes responsible for post-translational proteolytic processing, as the main example of Mpro. Ulferts, Mettenleiter and Ziebuhr [30] confirmed that the enzyme Mpro is a serine protease, and that it makes use of its catalytic triad Ser-His-Asp.

The need for validated and targeted therapeutic agents for the treatment of this disease led to the identification of an inhibitor based on mechanism (N3) followed by the determination of the crystalline structure of Mpro in complex with this compound, demonstrating the importance of rational screening studies in the search of new drugs with clinical potential [6].

The limonoids used in this study presented, in their majority, positions of interaction with Mpro relatively close to that of the drugs

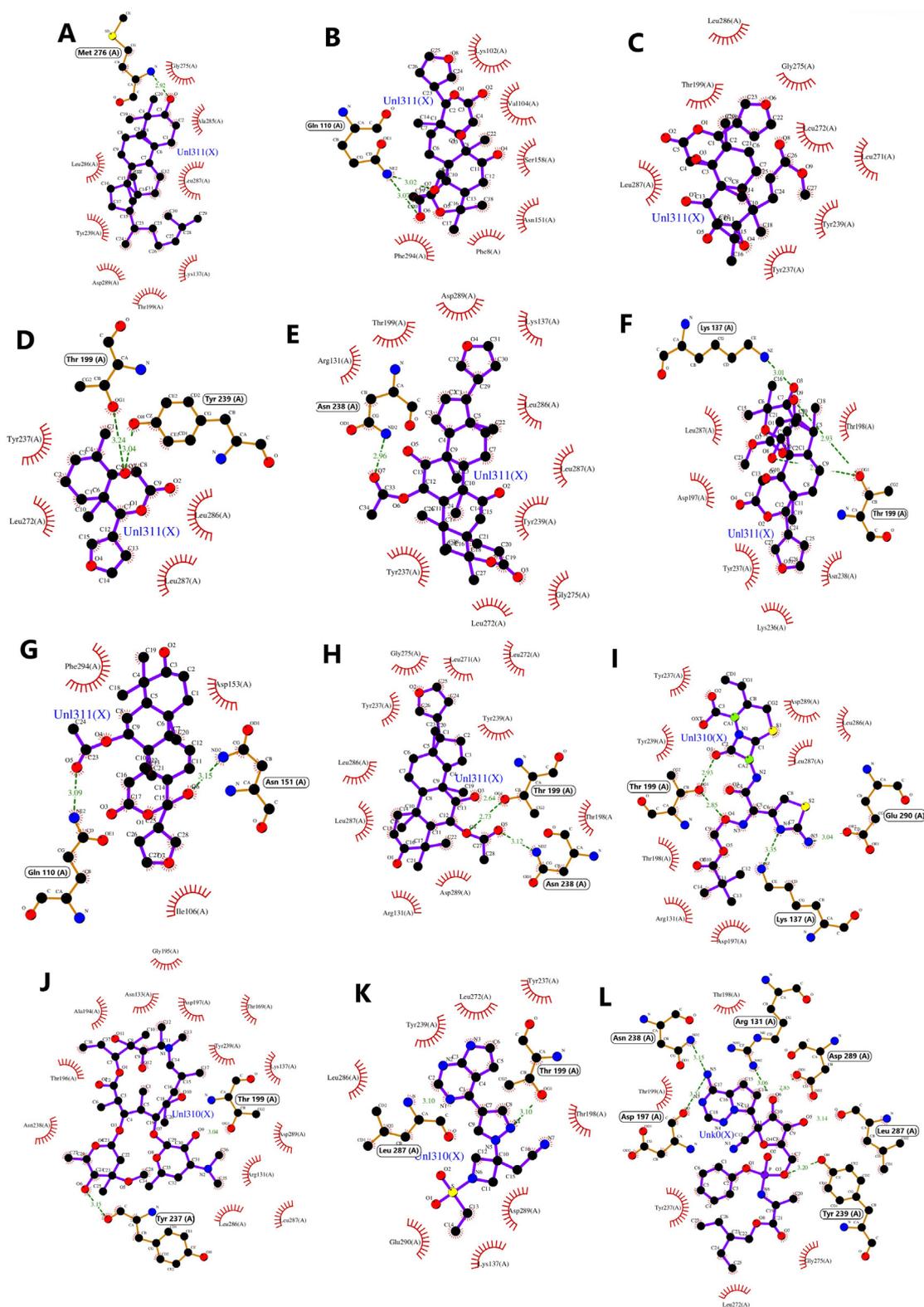


Fig. 3. Hydrogen interactions with some limonoids structures. Euphadienol (A), Limonine (B), Desacetilspatheлина (C), Calodemdrolid (D), Pedonina (E), [Harrisonin (F), Nimolicinol (G) and Deacetylnimokinol (H), Anakinra (I), Azithimycin (J), Baricitinib (K) and Remdesivir (L).

worked, with a greater emphasis on Thr199, highlighting CLD, HSN and DNM. These findings are promising, since *in silico* studies describe that RDS forms stable hydrogen bonds with residues from the same region, with a binding affinity around -8.2 kcal/mol, indicating potential as a therapeutic inhibitor [31]. In the present study, limonoids showed a higher interaction affinity than RDS.

Therefore, the results presented in the present work are innovative, since, among the studied molecules, there is practically no description of their biological activities in the literature, highlighting only the larvicidal and insecticide effects [9,32], as well neuroprotective activities in rat cortical cell culture [33]. In this context, terpenoids, in general, are a group of substances of natural origin with a variety of biological and

Table 2

Interaction distances (Å) between the compounds and the amino acid residues of SARS-CoV-2 Mpro.

RESIDUE	199A	287A	238A	131A	289A	239A	197A
Amino acid	THR	LEU	ASN	ARG	ASP	TYR	ASP
Limonoids							
Calodendrolide	3.24	3.23	5.16	7.05	6.39	3.04	6.51
6 α -O-acetyl-7-desacetylespathelin	3.07	3.60	5.03	6.37	5.29	3.41	6.83
6 α -O-acetyl-7-deacetylnimocinol	2.64	3.68	3.12	3.34	3.86	4.16	3.75
Harrisonin	2.70	3.73	4.22	3.53	4.24	4.85	4.06
Limonine	19.41	18.16	23.12	14.64	11.57	19.71	18.02
Nimolicinol	14.91	17.61	21.85	13.50	11.13	20.09	16.21
Pedonina	3.17	2.94	2.96	3.43	3.51	3.61	4.69
Euphadienol	3.48	3.44	6.58	4.21	3.75	3.64	5.09
Reference ligands							
Anakinra	2.85	3.46	5.03	2.94	3.36	4.50	3.38
Azithomycin	3.04	3.66	3.93	3.84	3.40	5.72	4.93
Baricitinib	3.10	3.10	5.77	3.99	3.38	3.12	4.20
Remdesivir	3.56	3.14	3.15	3.06	2.85	3.20	3.31

CLD – Calodendrolide; DSP - 6 α -O-acetyl-7-desacetylespathelin; DNM - 6 α -O-acetyl-7-deacetylnimocinol; HSN – Harrisonin; LMN – Limonin; NCL – Nimolicinol; PDN – Pedonin; EPD – Euphadienol.

pharmacological activities, including antiviral activity. Silvestrol (17, 24-epoxy-25-hydroxy-3-oxobaccharan-21-oic acid), for example, which is a triterpene that inhibits the enzyme RNA helicase involved in the translation of viral mRNA in human macrophages infected with Ebola virus, also inhibit the process of viral translation and replication in lung cells infected with coronavirus [8].

5. Conclusion

In conclusion, limonoids with important interactions with Mpro SARS-CoV-2 have been identified, indicating that these molecules are promising in the development of new drugs and/or biotechnological tools in the context of COVID-19 pandemic. The importance of in-depth studies of theoretical and medicinal chemistry associated with development in the health field, especially pharmaceuticals, is also emphasized, in the search for improved therapeutic options, with high efficacy and less toxicity.

Data availability

All data generated or analyzed during this study are included in this published article.

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Authors' contributions

Credit Author Statement: Conceptualization, Tiago Lima Sampaio; Data curation, Ramon Róseo Paula Pessoa Bezerra de Menezes; Formal analysis, Márcia Machado Marinho; Funding acquisition, Emmanuel Silva Marinho; Investigation, Victor Moreira de Oliveira; Methodology, Márcia Machado Marinho; Project administration; Resources; Software, Emmanuel Silva Marinho and Hécio Silva dos Santos; Supervision; Validation, Emmanuel Silva Marinho; Roles/Writing - original draft, Hécio Silva dos Santos; Writing - review & editing. Authorship Victor Moreira de Oliveira, Emanuel Paula Magalhães. All authors wrote the

first draft of the manuscript, as well as approved the final manuscript.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Consent to participate

All authors agree to participate.

Consent for publication

All authors agree to publish.

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References

- [1] Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J. Autoimmun.* 2020;109:102433. <https://doi.org/10.1016/j.jaut.2020.102433>.
- [2] Kemp SA, Collier DA, Datt RP, Ferreira IATM, Gayed S, Jahun A, Hosmillo M, Rees-Spear C, Mlcochova P, Lumb IU, Roberts DJ, Chandra A, Temperton N, Sharrocks K, Blane E, Modis Y, Leigh K, Briggs J, van Gils M, Smith KGC, Bradley JR, Smith C, Doffinger R, Ceron-Gutierrez L, Barcenas-Morales G, Pollock DD, Goldstein RA, Smielewska A, Skittrall JP, Gouliouris T, Goodfellow IG, Gkrania-Klotsas E, Illingworth CJR, McCoy LE, Gupta RK. SARS-CoV-2 evolution during treatment of chronic infection. *Nature* 2021. <https://doi.org/10.1038/s41586-021-03291-y>.
- [3] Garrido I, Liberal R, Macedo G. Review article: COVID-19 and liver disease—what we know on 1st May 2020. *Aliment. Pharmacol. Ther.* 2020. <https://doi.org/10.1111/apt.15813>.
- [4] WHO. Coronavirus Disease (COVID-19) Situation Report—160. 2020.
- [5] Bzówka AGM, Mitusińska K, Raczynska A, Samol A, Tuszyński JA. Structural and evolutionary analysis indicate that the sars-cov-2 mpro is a challenging target for small-molecule inhibitor design. *Int. J. Mol. Sci.* 2020;21.
- [6] Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y, Yu J, Wang L, Yang K, Liu F, Jiang R, Yang X, You T, Liu X, Yang X, Bai F, Liu H, Liu X, Guddat LW, Xu W, Xiao G, Qin C, Shi Z, Jiang H, Rao Z, Yang H. Structure of Mpro from COVID-19 virus and discovery of its inhibitors. *Nature* 2020:582. <https://doi.org/10.1038/s41586-020-2223-y>.
- [7] Jukić M, Janežič D, Bren U. Ensemble docking coupled to linear interaction energy calculations for identification of coronavirus main protease (3CLpro) non-covalent

- small-molecule inhibitors. *Molecules* 2020;25. <https://doi.org/10.3390/molecules25245808>.
- [8] Müller C, Schulte FW, Lange-Grünweller K, Obermann W, Madhugiri R, Pleschka S, Ziebuhr J, Hartmann RK, Grünweller A. Broad-spectrum antiviral activity of the eIF4A inhibitor silvestrol against corona- and picornaviruses. *Antivir. Res.* 2018; 150:123–9. <https://doi.org/10.1016/j.antiviral.2017.12.010>.
- [9] Garcez UC, W S, * Garcez FR, Silva LMGE, Sarmento. Substâncias de Origem Vegetal com Atividade Larvicida Contra *Aedes aegypti* 2013;5:363–93. <https://doi.org/10.5935/1984-6835.20130034>.
- [10] Pan YK. Molecular quantum mechanics: an introduction to quantum chemistry (Atkins, P.W.). *J. Chem. Educ.* 1971. <https://doi.org/10.1021/ed048pa294.2>.
- [11] Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* 1996;17:490–519. [https://doi.org/10.1002/\(SICI\)1096-987X\(199604\)17:5<490::AID-JCC1>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1096-987X(199604)17:5<490::AID-JCC1>3.0.CO;2-P).
- [12] Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J. Cheminf.* 2012. <https://doi.org/10.1186/1758-2946-4-17>.
- [13] Z J, et al. Structure of Mpro from COVID-19 Virus and Discovery of its Inhibitors. *BioRxiv*; 2020.
- [14] Shityakov S, Förster C. In silico predictive model to determine vector-mediated transport properties for the blood–brain barrier choline transporter. *Adv. Appl. Bioinforma. Chem.* AABC. 2014;7:23–36. <https://doi.org/10.2147/AABC.S63749>.
- [15] Marinho EM, Batista de Andrade Neto J, Silva J, Rocha da Silva C, Cavalcanti BC, Marinho ES, Nobre Júnior HV. Virtual screening based on molecular docking of possible inhibitors of Covid-19 main protease. *Microb. Pathog.* 2020;148:1–6. <https://doi.org/10.1016/j.micpath.2020.104365>.
- [16] Francisco FW, Castro Matos MG, Marinho EM, Marinho MM, Róseo Paula Pessoa Bezerra de Menezes R, Sampaio TL, Bandeira PN, Celedonio Fernandes CF, Magno Rodrigues Teixeira A, Marinho ES, de Lima-Neto P, Silva dos Santos H. In silico study of the potential interactions of 4'-acetamidochalcones with protein targets in SARS-CoV-2. *Biochem. Biophys. Res. Commun.* 2021;537:71–7. <https://doi.org/10.1016/j.bbrc.2020.12.074>.
- [17] Jukić M, Škrlić B, Tomšić G, Pleško S, Podlipnik Č, Bren U. Prioritisation of compounds for 3clpro inhibitor development on SARS-CoV-2 variants. *Molecules* 2021;26. <https://doi.org/10.3390/molecules26103003>.
- [18] Trott O, Olson AJ, Vina AutoDock. Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010;31:455–61. <https://doi.org/10.1002/jcc.21334>.
- [19] Shityakov S, Förster C. Silico predictive model to determine vector-mediated transport properties for the blood-brain barrier choline transporter. *Adv. Appl. Bioinforma. Chem.* 2014. <https://doi.org/10.2147/AABC.S63749>.
- [20] Imberty A, Hardman KD, Carver JP, Pérez S. Molecular modelling of protein-carbohydrate interactions. Docking of monosaccharides in the binding site of concanavalin A - *PubMed*. *Glycobiology* 1991;1:631–42.
- [21] Tao Q, Du J, Li X, Zeng J, Tan B, Xu J, Lin W, Chen X-L. Network pharmacology and molecular docking analysis on molecular targets and mechanisms of Huashi Baidu formula in the treatment of COVID-19. *Drug Dev. Ind. Pharm.* 2020;46:1345–53. <https://doi.org/10.1080/03639045.2020.1788070>.
- [22] Narkhede RR, V Pise A, Cheke RS, Shinde SD. Recognition of natural products as potential inhibitors of COVID - 19 main protease (mpro): in - silico evidences. *Nat. Products Bioprospect* 2020. <https://doi.org/10.1007/s13659-020-00253-1>.
- [23] da Silva Arouche T, Reis AF, Martins AY, S Costa JF, Carvalho Junior RN, Neto AMJC. Interactions between Remdesivir, ribavirin, favipiravir, galidesivir, hydroxychloroquine and chloroquine with fragment molecular of the COVID-19 main protease with inhibitor N3 complex (PDB ID:6LU7) using molecular docking. *J. Nanosci. Nanotechnol.* 2020;20:7311–23. <https://doi.org/10.1166/jnn.2020.18955>.
- [24] Vardhan S, Sahoo SK. In silico ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. *Comput. Biol. Med.* 2020;124:103936. <https://doi.org/10.1016/j.combiomed.2020.103936>.
- [25] Alves DR, Nunes M, De Sousa DS, Cristina I, Oliveira M, Marinho MM, De Moraes SM, Marinho ES. Virtual screening of natural curcumins and related compounds against SARS-CoV-2. *J. Comput. Biophys. Chem.* 2021;20:53–70. <https://doi.org/10.1142/S2737416521500046>.
- [26] Enmozhi SK, Raja K, Sebastine I, Joseph J. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach. *J. Biomol. Struct. Dyn.* 2021; 39:3092–8. <https://doi.org/10.1080/07391102.2020.1760136>.
- [27] Vijayakumar BG, Ramesh D, Joji A, Jayachandra prakasan J, Kannan T. In silico pharmacokinetic and molecular docking studies of natural flavonoids and synthetic indole chalcones against essential proteins of SARS-CoV-2. *Eur. J. Pharmacol.* 2020. <https://doi.org/10.1016/j.ejphar.2020.173448>.
- [28] Talluri S. Molecular docking and virtual screening based prediction of drugs for COVID-19. *Comb. Chem. High Throughput Screen.* 2021;24:716–28. <https://doi.org/10.2174/1386207323666200814132149>.
- [29] Daoud S, Alabed SJ, Dahabiyeh LA. Identification of potential COVID-19 main protease inhibitors using structure-based pharmacophore approach, molecular docking and repurposing studies. *Acta Pharm.* 2021;71:163–74. <https://doi.org/10.2478/acph-2021-0016>.
- [30] Ulferts R, Mettenleiter TC, Ziebuhr J. Characterization of bafinivirus main protease autoprocessing activities. *J. Virol.* 2011;85:1348–59. <https://doi.org/10.1128/jvi.01716-10>.
- [31] Naik VR, Munikumar M, Ramakrishna U, Srujana M, Goudar G, Naresh P, Kumar BN, Hemalatha R. Remdesivir (GS-5734) as a therapeutic option of 2019-nCoV main protease - in silico approach. *J. Biomol. Struct. Dyn.* 2020:1–14. <https://doi.org/10.1080/07391102.2020.1781694>.
- [32] Kiprop AK, Kiprono PC, Rajab MS, Kosgei MK. Limonoids as larvicidal components against mosquito larvae (*Aedes aegypti* Linn.). *Zeitschrift Fur Naturforsch. - Sect. C J. Biosci.* 2007;62:826–8. <https://doi.org/10.1515/znc-2007-11-1209>.
- [33] Jeong SY, Sang HS, Young CK. Neuroprotective limonoids of root bark of *Dictamnus dasycarpus*. *J. Nat. Prod.* 2008;71:208–11. <https://doi.org/10.1021/np070588o>.