REVIEW ARTICLE

Enzymes in the time of COVID-19: An overview about the effects in the human body, enzyme market, and perspectives for new drugs

Luana Xavier Soares Gomes Moura Fé[1] |
Eliane Pereira Cipolatti[1,2] | Martina Costa Cerqueira Pinto[3,4] |
Suema Branco[5] | Fábio César Sousa Nogueira[3] |
Gisela Maria Dellamora Ortiz[6] | Anderson de Sá Pinheiro[3] |
Evelin Andrade Manoel[1,3]

[1]Departamento de Biotecnologia Farmacêutica, Faculdade de Farmácia, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ)—Cidade Universitária, Rio de Janeiro, Rio de Janeiro, Brazil

[2]Departamento de Engenharia Química, Instituto de Tecnologia, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil

[3]Departamento de Bioquímica, Instituto de Química, Centro de Tecnologia (CT), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil

[4]Chemical Engineering Program, Instituto Alberto Luiz Coimbra de Pós-graduação e Pesquisa de Engenharia (COPPE), Centro de Tecnologia (CT), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil

[5]Biofísica Ambiental, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Rio de Janeiro, Brazil

[6]Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ)—Cidade Universitária, Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence
Evelin Andrade Manoel. Departamento de Biotecnologia Farmacêutica, Faculdade de Farmácia, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ)— Avenida Carlos Chagas Filho, 373. Cidade Universitária, CEP: 21.941-902, Rio de Janeiro, RJ, Brazil,
Email: eamanoel@pharma.ufrj.br

Abstract

The rising pandemic caused by a coronavirus, resulted in a scientific quest to discover some effective treatments against its etiologic agent, the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). This research represented a significant scientific landmark and resulted in many medical advances. However, efforts to understand the viral mechanism of action and how the human body machinery is subverted during the infection are still ongoing. Herein, we contributed to this field with this compilation of the roles of both viral and human enzymes in the context of SARS-CoV-2 infection. In this sense, this

overview reports that proteases are vital for the infection to take place: from SARS-CoV-2 perspective, the main protease (M^{pro}) and papain-like protease (PL^{pro}) are highlighted; from the human body, angiotensin-converting enzyme-2, transmembrane serine protease-2, and cathepsins (CatB/L) are pointed out. In addition, the influence of the virus on other enzymes is reported as the JAK/STAT pathway and the levels of lipase, enzymes from the cholesterol metabolism pathway, amylase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and glyceraldehyde 3-phosphate dehydrogenase are also be disturbed in SARS-CoV-2 infection. Finally, this paper discusses the importance of detailed enzymatic studies for future treatments against SARS-CoV-2, and how some issues related to the syndrome treatment can create opportunities in the biotechnological market of enzymes and the development of new drugs.

KEYWORDS

biomarkers, COVID-19, enzyme market, SARS-CoV-2, target enzymes

1 | INTRODUCTION

At the end of 2019, the world was impacted by a novel infectious respiratory disease caused by a coronavirus. Despite the first case being reported in Wuhan, Hubei province, China,[1] the virus spread rapidly to other continents.[2] This disease was named by the World Health Organization as Coronavirus Disease 2019 (COVID-19)[3] and, on January 30th, 2020, it was declared as a Public Health Emergency of International Concern.[4] Then, on March 11th, 2020, it achieved pandemic status.[2]

The COVID-19 etiologic agent is the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2),[5] previously known as WH-Human 1 Coronavirus and 2019-nCoV.[6] This pathogen is an enveloped single-stranded positive-polarity RNA virus that belongs to the Coronavirus family,[7] more specifically to the Betacoronavirus genus,[5] subgenus Sarbecovirus.[6] The SARS-CoV-2 genome consists of approximately 30 kb and it is translated into four structural proteins and several nonstructural proteins (nsp).[6] The structural proteins are the spike (S), envelope (E), membrane (M) and nucleocapsid (N).[6] The nsp have different functions,[8] but among them there are the viral proteases main protease (M^{pro}) and papain-like protease (PL^{pro}),[9] respectively nsp5 and nsp3.[10]

SARS-CoV-2 and Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) genomes share 89.1% genome identity.[6] The SARS-CoV itself was responsible for the pandemic that was originated in China in 2002–2003.[11] Even though SARS-CoV-2 seems to be easier spread among humans than SARS-CoV,[5] both viruses seem to use angiotensin-converting enzyme-2 (ACE2) to invade human cells[12] and to cause similar symptoms,[6,11] such as acute

respiratory distress syndrome (ARDS) and pneumonia.[5] However, other symptoms can be triggered as well, such as fever,[5] chest tightness, unproductive cough, headache,[6] myalgia or fatigue, lymphopenia and dyspnea.[13]

Despite the initiated quest against SARS-CoV-2 and the consequent abundance of papers, to the best of our knowledge the information about the involvement of viral and human enzymes in the course of the infection is dispersed, which may hamper the discovery of a specific drug to treat COVID-19. De La Fuente et al.[14] discussed enzyme therapeutics and their challenges, including as treatment for SARS-CoV-2 infection, pointing out different enzymes, such as RNases, catalase, binase and fungal proteases, as potential therapies. Paulsson-Habegger, Snabaitis, Wren[15] considered only human enzymes related with SARS-CoV-2 entrance in host cell and drugs that can inhibit them. The review presented by Majerová and Novotný[16] does contribute to that field, but its approach is solely on viral proteases, addressing their structures and inhibitors. However, Majerová and Novotný[16] lack a discussion on human enzymes involved in SARS-CoV-2 infection, and those three papers do not present an overview on how the enzyme market may benefit from the production of specific medicines against the infection, and from the production of tools to detect the enzymatic alterations that SARS-CoV-2 causes on the human body. Also, those three papers do not mention some human enzymes that can, and others hypothesized to, play a role in SARS-CoV-2 infection. These enzymes will be addressed in the present work.

Therefore, in this paper, we aimed to review the influence of SARS-CoV-2 enzymes during the infection process. We intended to discuss the role of some human and viral enzymes of COVID-19 and to give a landscape of enzymatic alterations in the host during SARS-CoV-2 infection. Moreover, some enzymes that have been poorly studied in SARS-CoV-2 infection, but may be important in this context, are addressed here for future analysis. Suggestions for possible treatments to COVID-19 are also reported in this paper, although a definitive drug protocol has not been established yet by medical boards. Suggestions for future steps in understanding the viral mechanism of action and the severity of the infection will also be presented in this paper.

Interestingly, glycogen synthase kinase-3 (Gsk-3) was pointed out to be associated with SARS-CoV-2 infection as it phosphorylates the N protein, and Gsk-3 inhibitors, such as lithium carbonate, which can impair viral replication in lung cells.[17] However, once lithium has a narrow therapeutic index, other Gsk-3 inhibitors may be considered as well, such as 9-ING-41,[18] an ATP-competitive inhibitor, and tideglusib, a non-ATP competitive inhibitor that belongs to the thiadiazolidinone class of drugs.[19] Previously, Gsk-3 was supposed to act in SARS-CoV-2 infection due to its role in SARS-CoV disease, including viral transcription, inhibition of host translational events, upregulation of cytokines, and support of oxidative stress.[19] Nonetheless, Gsk-3 has not received the attention it deserves for just a few papers mention that enzyme,[17–20] and therefore this field could benefit from more investigation and will not be further discussed here.

Furin is a protease related to coronavirus cell entrance as it is responsible for the cleavage of SARS-CoV-2 S protein in lung cells and primary human airway epithelial cells in a transmembrane serine protease-2 (TMPRSS2)-expression dependent manner.[21] However, the physiological consequences of inhibiting that enzyme are not known yet,[15] which suggests that this via of SARS-CoV-2 entrance should be more deeply investigated, and thus it will not be further explored in this paper.

As expected, the current economic crisis caused by the SARS-CoV-2 pandemic affected the global market of enzymes. However, whilst the impact of COVID-19 exhibited negative effects in several economic areas, the future of the enzyme market appears very promising.[22] Therefore, our study also aims at evaluating how the necessity of a treatment for COVID-19 can create opportunities to the biotechnological market of enzymes and to a new era in the development of new drugs.

2 | COVID-19 AND ENZYMES IN THE LITERATURE

The pandemic started a scientific quest to better understand the effects of COVID-19 and all aspects related to it, which has caused a significant rise in the number of publications in the recent period. For example, a search on the Scopus platform using "COVID-19" in "all fields" as the keyword, on June 9th, 2022, resulted in 318,780 scientific

documents. Among those, 164,745 were published in 2021; 67,967 are already estimated to be published in 2022, 21 in 2023, and two (2) in 2024. On the other hand, using the same keyword, the Science Direct platform resulted in 133,119 scientific documents, being 78 planned for publication in 2023. Although several aspects are addressed within these extensive studies described in the literature, no study has been found so far aiming to understand the effects of SARS-CoV-2 infection on human enzymes.

By narrowing the search using the keywords "COVID-19" AND "enzyme" (June 9th, 2022), on Scopus, 17,377 results were obtained including all types of documents, 2962 of which are planned for publication in 2022 and six (6) estimated in 2023. Figure 1A presents an overview of the distribution of such publications in distinct countries. Two of them stand out: the United States of America (USA) and China, which together contributed to 30,47% of all scientific publications in this field. In total, 163 countries appeared on the list, highlighting USA, China, India, Italy, United Kingdom, Germany, Iran, France, Canada, Spain and Brazil. A search was also made for affiliations that have contributed the most to the subject to date (Figure 1B). Chinese Academy of Sciences (China) is highlighted with 302 publications, followed by Inserm (France) and Harvard Medical School (USA) with 286 and 285 works, respectively, from a total of 163 organizations found in this platform.

Using the patentinspiration.com platform and the keyword "COVID-19," June 9th, 2022, exactly 6,363 patents were found, being 635 (2020), 3264 (2021) and 2464 (2022). Among the applicant countries, the United States of America and China again stand out, with 3719 and 495 patents, respectively. As an applicant, the "University of California" (USA) and "AzothBio Inc." (South Korea) stands out both with 36 patents, followed by the "Institut National de la Santé et de la Recherche Medicale" (France) and "Massachusetts Institute of Technology" (USA) with 23 and 18 patents, respectively. Using the same platform with "COVID-19" and "Enzyme" as keywords, 1,256 patents were found (63 in 2020, 698 in 2021 and 495 in 2022). Most of them comprise "antivirals" followed by "medicinal preparations with active compounds."

FIGURE 1   An overview of COVID-19 in the world. All data was based on Scopus platform using the keywords "COVID-19" AND "Enzyme" (June 9th, 2022). (A) Countries that published on the topic. (B) Affiliations with its respective number of publications found in all period (Due to the new theme, results appeared only between 2019 and 2023). (C) Enzymes that presented the greatest number of occurrences in the search. The Figures (A) and (B) were obtained by excel. The Figure (C) data was obtained by VosViewer [Color figure can be viewed at wileyonlinelibrary.com]

It is also expected that many studies will be published in the upcoming years relating enzymes to COVID-19, since the pandemic is still ongoing. There is increasing use of enzymes for COVID-19 diagnosis and the effects of SARS-CoV-2 infection on human enzymes are currently not entirely understood. Generally, research groups compete with each other for the originality in the publication of articles or patents, but in the pandemic scenario the collective contributions and sharing of credits are acquiring great strength.[23]

Using the software VOSviewer 1.6.11 (Leiden University, Leiden, Netherlands), it was possible to extract keywords from the titles, abstracts and citation contexts contained in the Scopus platform. Then it was possible to identify all enzymes published (Figure 1C). In our search, we used "co-occurrence" as the type of analysis, "all keywords" as the unit of analysis, "full counting" as counting method, and a minimum number of occurrences of 10, resulting in 862 keywords that were analyzed. Among those, only the enzymes were used to generate Figure 1C. Enzymes mentioned as "others" include: "alkaline phosphatase," "main protease," "transmembrane protease serine 2," "protein kinase B," "RNA replicase," "liver enzyme," "viral enzyme," "microsomal aminopeptidase," "creatine kinase," "cysteine proteinase," "γ-glutamyltransferase," "hydrolase," and "3c-like proteinase." Some of these enzymes will be discussed throughout the text.

It is important to highlight that some enzymes play crucial roles as "entrance tickets" to SARS-CoV-2 infection (Figure 2). Alongside other viruses, SARS-CoV-2 uses host enzymes to bind to cells or suppress enzymatic/protein pathways that are important to the proper functioning of the human body.[7,24] Thus, the study of enzymes involved in the molecular mechanisms leading to COVID-19 is essential for the development of new treatment and diagnostic strategies. This subject will be discussed in the next sections.

# 3 | ENZYMES ARE ENTRANCE TICKETS FOR SARS-COV-2 TO INVADE THE HUMAN BODY AND CAN BE USEFUL DRUG TARGETS

As seen in Figure 2, some human proteases, such as ACE2,[5–7,12] TMPRSS2 and cathepsins B and L (CatB/L),[25] are vital to SARS-CoV-2 entry. Two viral proteases, M^{pro} [26] and PL^{pro},[27] are needed to process the viral genome and PL^{pro} can be also used to evade the host immune system.[28] Besides, kinases can be affected by SARS-CoV-2 infection, such as the kinases from the JAK/STAT pathway, which is a kinase cascade responsible for cell signaling in immunological processes.[29,30] In this section, those human and viral enzymes are discussed, as well as some enzymes that can play a role in COVID-19 but have not received much attention so far. Among the enzymes that can be deeper studied are Cullin ligase[31]; ubiquitin specific peptidase 13 (USP13)[32]; the cytidine deaminases apolipoprotein B messenger RNA (mRNA) editing catalytic polypeptide-like subunit 3 (APOBEC3) and adenosine deaminase acting on RNA (ADAR)[33]; and serine peptidase inhibitors (SERPINs) A1,[34] A3 [35] and A5,[36] which are also known respectively as α$_1$-antitrypsin,[37] α$_1$-antichymotrypsin[37] and protein C inhibitor,[37] and have serine or cysteine activity.[38] Also in this section, those enzymes are shown as possible drug and prototype drug targets.

3.1 | Host enzymes

3.1.1 | Angiotensin-converting enzyme-2

3.1.1.1 | _General aspects_
The ACE2 is expressed in a wide range of human tissues and organs, as seen in Table 1.[24,39] This enzyme is a type I membrane protein[7] of 92.4 kDa,[40] as seen in Table 1; but a soluble form also circulates in the blood at low levels.[41] The transmembrane ACE2 has an extracellular _N_-glycosylated N-terminal domain, which houses the active site, and a small intracellular C-terminal domain, whereas the soluble form does not have the cytosolic and transmembrane regions, as a result of a physiological shedding event by tumor necrosis factor-α convertase (ADAM17).[42]
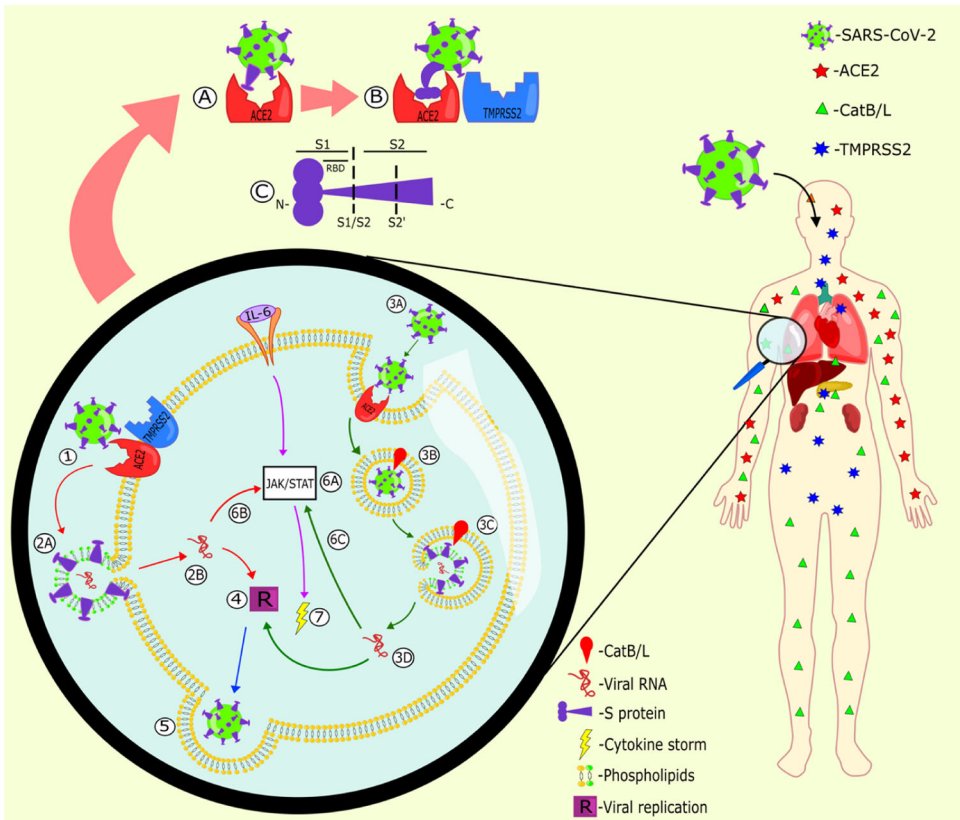
FIGURE 2 Simplified representation of the mechanism of action of SARS-CoV-2. Initially, the Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) approaches the cell membrane (1) and it binds to the angiotensin converting enzyme-2 (ACE2; colored in red) **(pink arrow, step A)** through the receptor binding domain (RBD) of the viral spike (S) protein. The S protein suffers a conformational change and it is cleaved by the transmembrane serine protease-2 (TMPRSS2; colored in blue) **(pink arrow, step B)**, when this protease is expressed in the cell. The S protein has two cleavage sites, represented by the dotted lines, S1/S2, which is between the S1 and S2 domains; and S2′, which is within S2 domain; also, the RBD is housed in the S1 domain **(pink arrow, step C)**. After binding ACE2 and being cleaved by TMPRSS2, the virus fuses with the cell membrane (2A) and and the viral RNA is released in the cytosol (2B). Alternatively, the virus can enter the cell through endocytosis (3A). In the endosome, the viral particle is cleaved by cathepsins B and L (CatB/L) (3B). The viral particle fuses with the endosome membrane (3C) and the viral RNA is released in the cytosol (3D). After the release of the viral RNA, either by previous binding of the viral particle to ACE2 or by previous endosome encompassing, the virus undergoes the replication step (4), in which it subverts the cell machinery (not shown). After this step and the synthesis of the viral polyproteins (pp) pp1a and pp1ab, the viral proteases main protease (M^{pro}) and papain-like protease (PL^{pro}) play important roles in releasing themselves by autoproteolytic activity and in processing the central and the C-proximal region of pp1a/pp1ab (M^{pro}) and the N-proximal region of pp1a/pp1ab (PL^{pro}) (not shown). After replication, a new viral particle is generated and it is released from the cell (5). The presence of the viral RNA detected in other cells elicits the release of cytokines, such as interleukin-6 (IL-6, not discussed in this paper), in the body. IL-6 binds to its cell membrane receptor, it activates the JAK/STAT pathway (6A) and it promotes the cytokine storm (7) in the targeted cells. In parallel, the release of viral RNA in the cytosol **(steps 1, 2A, and 2B, or steps 3A, 3B, and 3C)** can also directly activate the JAK/STAT pathway in the same cell (6B and 6C), and then it promotes the cytokine storm (7). ACE2, catB/L and TMPRSS2 are ubiquitously expressed in the body. TMPRSS2, transmembrane serine protease-2 [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Host enzymes involved in SARS-CoV-2 infection that can be useful drug targets

	Host enzymes			
Features	Angiotensin-converting enzyme-2 (ACE2)	Transmembrane protease serine protease-2 (TMPRSS2)	Cathepsins B and L (CatB/L)	JAK/STAT pathway
Location in Organs and Tissues	Vascular system, heart, kidneys, upper airway, liver, pancreatic islets, gut, retina, central nervous system, monocytes and macrophages	Nose; heart; intestines, both small and large; esophagus, testis and kidney; which may jeopardize the respiratory, digestive and reproductive systems	Ubiquitously expressed in the human body	Ubiquitously expressed in the human body
Cellular Localization	Cell membranes (type I membrane protein, ~92.4 kDa), Circulates in blood in low levels	Cell membranes (type II membrane protein, ~70 kDa)	Lysosomes	Associated to membrane receptor's after binding of extracellular messenger (JAK) Associated to membrane receptor's after binding of extracellular messenger Nucleus and mitochondrion (STAT)
Enzymatic Function	Carboxypeptidase (PXP-hydrophobic/basic), Zinc metalloprotease (HEXXH, with a glutamate residue that acts as a third zinc-binding point)	Serine protease Calcium-binding	Both cathepsins are cysteine protease They belong to the papain-like protease family Cathepsin L (~34 kDa) is an endopeptidase Cathepsin B (~38 kDa) is both exo- and endopeptidase	Tyrosine kinase cascade
Physiological Role(s)	Convert angiotensin (Ang) II to Ang-(1-7), Convert Ang I to Ang-(1-9), Chaperone of neutral amino acid transporter B0AT1	Protein turnover and processing, such as activating zymogens and hormones, Acts in epidermal and cardiac homeostasis	Digestion, tissue remodeling, blood coagulation, fertility, inflammatory responses and apoptosis, Is highly expressed in prostate and colon carcinomas	It is a signaling tyrosine kinase cascade that communicates from membrane to nucleus, It is flared by type I IFN in viral infections and leads to the production of Interferon-stimulated genes (ISG), which upregulates antiviral proteins, release of cytokines and recruitment of immune cells JAK-2 mediates tyrosine phosphorylation of nuclear histones

Note: In the row "Physiological Role(s)", "Cleavage of ACE2," appears at the top of the TMPRSS2 cell.

TABLE 1 (Continued)

	Host enzymes			
Features	Angiotensin-converting enzyme-2 (ACE2)	Transmembrane protease serine protease-2 (TMPRSS2)	Cathepsins B and L (CatB/L)	JAK/STAT pathway
Structure	ACE homologue, Homodimer, C-terminal ends in a transmembrane helix and in a cytosolic tail, in a collectrin-like domain (CLD), N-terminal is extracellular and contains a claw-like peptidase domain (PD) and the catalytic pockets, There is evidence that ACE2 has two conformations, named opened and closed ones, respectively with the two PD separated and close to each other	It has three functional domains, named LDL-receptor class A (LDLRA), at the N-terminal; scavenger receptor cysteine-rich (SRCR); serine-protease S1 (PS1), at the C-terminal, The LDLRA and the N-terminal are not completely structured. Besides, some residues on a loop that connects the N-terminal to SRCR are are responsible for binding to calcium ions	It has been seen for cathepsins that the active site amino acid residues are conserved, The cathepsin L, an endopeptidase, for example, counts on Cys25 and His163 in its active pocket. Its structure is organized in two domains, named as the left (L) and right (R) ones. The former has three alpha-helices and the latter has a beta-barrel shape, with the front of a coiled structure and the top closed by an alpha-helix. The cathepsin L catalytic cysteine and histidine are housed on the top of the sheet that forms the barrel	The JAKs have four domains: FERM, which is responsible for protein binding and has three subdomains similar to ubiquitin, CoA binding and pleckstrin homologyphosphotyrosine binding domains; SH2, which binds to phosphotyrosine; pseudotyrosine kinase, which has regulatory function; and tyrosine kinase, responsible for the catalytic activity The STATs have six domains: oligoamino terminal, responsible for oligomerization; coiled-coil, responsible for protein interaction; DNA binding; linker; SH2, which binds to phosphotyrosine; transactivation domain, which has SUMOylation function, responsible for STAT and transcription regulation, targeting nuclear proteins and protein interaction
Role in SARS-CoV-2 Infection	The PD is targeted by the receptor binding domain (RBD, ~21 kDa) of viral trimeric glycosylated S protein (~180 kDa, not considering 19 amino acids located in the tail of the S protein), and thus, ACE2 acts as anchorage point so that the virus can invade the cells	Processing the viral S protein in two points after it binds to ACE2	Activation of the viral S protein	The virus is supposed to inhibit the pathway, once that SARS-CoV inhibits STAT-1; It is supposed to flare an exacerbated immune response, as MERS-CoV

(Continues)

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TABLE 1 (Continued)

| Features | Host enzymes | | | |
	Angiotensin-converting enzyme-2 (ACE2)	Transmembrane protease serine protease-2 (TMPRSS2)	Cathepsins B and L (CatB/L)	JAK/STAT pathway
Biotechnological Approach	The interaction between viral RBD and host ACE2 downregulates the latter (apart from pancreas), which leads to an ACE/ACE2 imbalance and consequently to Ang II accumulation. Pharmacological approaches seek to restore the balance, but in some cases there is controversy. The approaches include use of ACE inhibitors; angiotensin II receptor blockers; Ang-(1-7) agonists; recombinant ACE2; ACE2 peptidomimetics; soluble ACE2; ACE2 fused to Fc segment of antibodies; monoclonal antibodies; ensovibep (a DARPin-based molecule that passed phase 2 clinical trial); ACE2 inhibitors	The proteolytic cleavage of the S protein from SARS-CoV-2 is an essential step for the infection course, so it can be a therapeutic approach of interest. An example of TMPRSS-2 inhibitor would be camostat mesylate, Other drugs were tested in silico, such as paritaprevir, daclatasvir, ombitasvir, eprosartan, lisuride, However, clinical data about the use of TMPRSS-2 inhibitors against SARS-CoV-2 still need to be done	The activation of the S protein from SARS-CoV-2 is an essential step for the infection course, so it can be a therapeutic approach of interest. An example of CatB/L inhibitor would be E-64-, However, it is important to remember that the use of CatB/L inhibitors against SARS-CoV-2 may lead to off target inhibition, for the drug must access the intracellular environment to find CatB/L	To diminish the exacerbated immune response, baricitinib, an inhibitor of JAK-1 and JAK-2 could be administered, but this could also reduce the protection against SARS-CoV-2

Abbreviations: ACE2, angiotensin-converting enzyme-2; Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1-7), angiotensin-(1-7); Ang-(1-9), angiotensin-(1-9); CatB/L, cathepsins B and L; CLD, collectrin-like domain; DARPin, designed Ankyrin repeat protein; ISG, interferon-stimulated genes; JAK/STAT pathway, Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) signaling pathway; L, left; LDLRA, LDL-receptor class A; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; PD, peptidase domain; PSI, serine-protease S1; R, right; RBD, receptor binding domain; S protein, spike protein; SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus 2; SRCR, scavenger receptor cysteine-rich; TMPRSS2, transmembrane protease serine protease-2.

The ACE2 shows carboxypeptidase activity[40] and cleaves Pro-X-Pro-hydrophobic/basic amino acid sequence,[43] as seen in Table 1. Structurally, ACE2 is a homodimer[7] and has a single zinc-binding domain (HEXXH). ACE2 has a homologue, ACE, which is a peptidyl-dipeptidase[40] responsible for converting angiotensin I to angiotensin II.[41] Differently from ACE2, ACE has two zinc-binding domains, which in this case seem to have catalytic activity rather than structural function.[44] Both ACE and ACE2, however, seem to have a third binding point to the zinc ion, occupied by a glutamate residue, corresponding to Glu402 for ACE2,[40] and to Glu987 and possibly Glu389 for ACE[44] (Table 1).

Physiologically, ACE2 is responsible for converting angiotensin I (Ang I) and angiotensin II (Ang II) to angiotensin-(1–9) (Ang-(1–9)) and angiotensin-(1–7) (Ang-(1–7)), respectively.[40,41] ACE converts Ang I into Ang II, mainly in the lungs.[45] Indeed, the ACE/ACE2 system, which also involves other enzymes, is present in different tissues and organs, but it is also a circulating system.[46] Ang II is important as a local and systemic blood pressure and homeostasis regulator.[46] Then, both ACE2 and ACE regulate hemodynamics in the body, counterbalancing the activity of each other through the availability of their substrates.[45] In addition, ACE2 plays several other physiological roles (Table 1).[7,40,41]
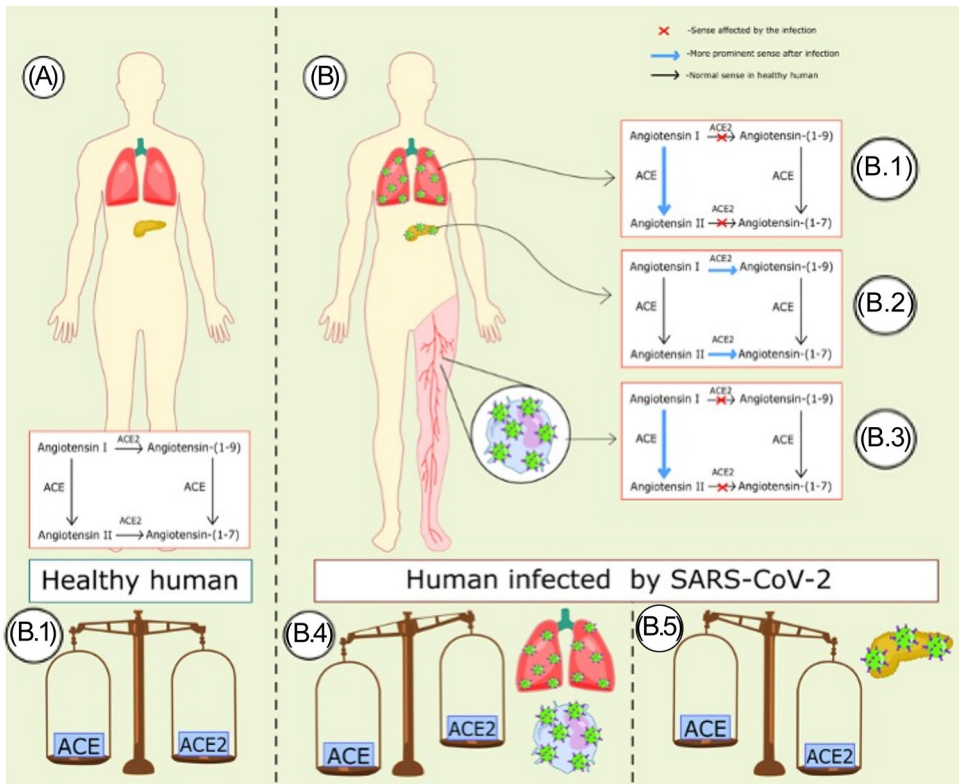
3.1.1.2 | In SARS-CoV-2 infection

From the SARS-CoV epidemic, it was known that the virus targets ACE2.[47] Consequently, ACE2 was the first enzyme studied in the context of SARS-CoV-2 infection.[5–7,12] Figure 2 shows a simplified representation of the role played by ACE2 in SARS-CoV-2 infection. Indeed, the peptidase domain (PD) of ACE2 is recognized by the receptor binding domain (RBD) of the S protein of both SARS-CoV[48] and SARS-CoV-2[5,7,49] (Table 1; Figure 2).

The interaction surface between ACE2 and the RBDs of SARS-CoV and SARS-CoV-2 is similar and is mostly mediated by electrostatic interactions. In addition, there is a stronger interaction between ACE2 Met82 and SARS-CoV-2 Phe486 than it is between ACE2 and Leu472 in SARS-CoV.[7] Moreover, regarding SARS-CoV-2, the interaction between RBD and PD is through an N-linked glycan on Asn90 of ACE2,[50] which is lacking in the case of SARS-CoV.[50] Then, the dissociation of the complex SARS-CoV-2 and ACE2 is more difficult than the one between SARS-CoV and ACE2.[50] Therefore, ACE2 binding to the S protein of SARS-CoV-2 is approximately 10–20-fold stronger than that of SARS-CoV, which can be related to the more efficient dissemination of SARS-CoV-2 in humans.[5]

Interestingly, SARS-CoV-2 does not use other coronaviruses receptors, such as aminopeptidase N (APN) and dipeptidyl peptidase 4 (DPP4).[12] In fact, in different species, except for mice, SARS-CoV-2 can only enter cells that express ACE2.[12] A similar profile was already seen for SARS-CoV and its deficiency to bind to ACE2 from mice and rats, which causes a less efficient infection.[51] Indeed, ACE2 from rats houses a glycosylation at Asn82, which would sterically prevent the interaction with SARS-CoV S protein.[48] In humans, however, there is a methionine in the position 82 of ACE2,[48] and the interaction of the SARS-CoV S protein with human ACE2 would therefore be less hindered. This structural difference could guide the rational design of potential inhibitors.

In SARS-CoV infection, the S protein competes with Ang II for ACE2, which blocks ACE2 activity and causes its downregulation in the membrane.[52] Previously, downregulation of surface ACE2 was seen for both human and mouse enzymes overexpressed in cells treated with a recombinant SARS-CoV S protein, and SARS-CoV S protein also led to an accumulation of Ang II in lungs of mice.[53] Once ACE2 physiological function of negatively regulating Ang II is compromised, SARS-CoV infection results in severe lung failure and lethality.[53]

The influence of SARS-CoV-2 on ACE2 levels is controversial. On the one hand, pancreatic levels of ACE2 increase in the presence of the virus (Figure 3), which can cause pancreatic injury.[54] Ang II is converted by ACE2 into Ang-(1–7)[45] and Ang-(1–9) can be converted into Ang-(1–7) by ACE.[55] In pancreatic cells, Ang-(1–7) is responsible for promoting insulin sensitivity and secretion, and therefore it increases glucose uptake.[56] Then, in COVID-19, the exacerbated activity of ACE2 in the pancreas[54] probably increases Ang-(1–7) level, whose activity would then be more prominent. The more prominent Ang-(1–7) activity of increasing insulin secretion may cause insulin resistance, leading pancreas to injury and maybe even to diabetes in SARS-CoV-2 patients. It has been

FIGURE 3 Simplified representation of the ACE/ACE2 relationship in a healthy person and in a SARS-CoV-2 infected patient. (A) In a healthy individual, angiotensin converting enzyme (ACE) and angiotensin converting enzyme-2 (ACE2) regulate each other's activity, through the availability of their substrates; (A.1) balanced ACE/ACE2 relationship, with both enzymes in basal levels. (B) In a SARS-CoV-2 patient, there is an imbalance in ACE/ACE2, due to downregulation of ACE2 in the lungs (B.1 and B.4), and monocytes (B.3 and B.4); and upregulation of ACE2 in pancreas (B.2 and B.5). This imbalance leads to more prominent activities of the other enzymatic axes (blue arrows in B.1, B.2, and B.3) and worsens the systemic effects of COVID-19. COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2 [Color figure can be viewed at wileyonlinelibrary.com]

observed that SARS-CoV-2 infection in pancreatic lineages can upregulate the insulin resistance pathway and downregulate the glucagon signaling pathway, due to increased apoptosis.[57] Chemokines, such as *CCL2*, *CXCL5* and *CXCL6*, and cytokines are upregulated in pancreatic lineages as well, contributing to injury, similarly to lung autopsy samples from COVID-19 patients.[57] Previously for SARS-CoV, It was seen that the virus causes damage in pancreatic islets, leading to acute diabetes.[58]

On the other hand, the binding of the viral S protein to ACE2 in lung cells leads to ACE2 downregulation, which guides the human body to higher levels of Ang II (Figure 3), and to lung vascular permeability and lung damage.[59] Regarding SARS-CoV-2, it was also seen that in circulating blood cells, mainly monocytes, there is ACE2-downregulation (Figure 3), which is caused by a reduction in its mRNA, both for the transmembrane and soluble forms, leading to the accumulation of Ang II in the organism of SARS-CoV-2 patients. However, a bigger cohort is still needed to confirm those data.[60]

The alteration in ACE2 levels[54,57,59,60] caused by the interaction between the SARS-CoV-2 S protein and ACE2 is pointed out to cause an imbalance of ACE/ACE2 in the body[61,62] (as seen in Figure 3). Consequently,

the cardiovascular system, the inflammatory and fibrotic pathways, the insulin secretion and the regulation of reactive oxygen species production are compromised.[62]

Despite the controversial influence of SARS-CoV-2 on ACE2, it has been suggested that reduction of the ACE/ACE2 imbalance may reduce morbidity and mortality in COVID-19 patients.[61] This reduction can be achieved in five ways, as seen in Table 1.

The first way is by inhibiting ACE.[63] As previously discussed, ACE is the counterpart of ACE2 and they regulate each other by the availability of their substrates.[45] Once that ACE2 is downregulated in COVID-19, the effects of ACE are exacerbated.[45] Thus, inhibiting ACE could restore ACE/ACE2 balance by promoting Ang I accumulation.[45] However, a multi-center phase 2 clinical trial in the United States, comprising symptomatic COVID-19 patients that were not previously taking ACE inhibitors nor Ang II receptor blockers, has shown that the administration of the ACE inhibitor losartan did not reduce hospitalizations nor reduced viral load.[64] Therefore, deeper studies regarding ACE inhibitors should be conducted in COVID-19 patients before clinical recommendation, to evaluate if the drugs do improve outcomes or not.

The second way comprises reducing Ang II activity, by blocking Ang II receptors[65] or using soluble ACE2.[66] Once that, apart from pancreas,[54] in SARS-CoV-2 infection ACE2 is downregulated, its substrate, Ang I, accumulates in the body.[45] Ang I will then be converted by ACE into Ang II, generating high levels of the latter.[45] Ang II activity is responsible for proinflammatory and profibrotic responses in the body.[67] Then, blocking Ang II receptors will lead to Ang II accumulation and consequently there will be a negative feedback to Ang II production.[45] Moreover, using Ang II receptor blockers can favor the conversion of Ang II to Ang-(1–7).[45] Soluble ACE2 can also be used to reduce Ang II as the body will be provided with exogenous ACE2 molecules, which will be able to act in place of endogenous ACE2.[66] Besides, currently a phase 3 study is being conducted in India and Australia to investigate if Ang II receptor blockers can reduce COVID-19 severity among patients at high risk.[68]

The third way is by stimulating molecules activated by ACE2.[62] This stimulation intends to counterbalance, directly or indirectly, the elevated level of Ang II, which is in excess in SARS-CoV-2 infection due to the unavailability of transmembrane ACE2.[66] One of the molecules activated by ACE2 is Ang-(1–7), which has a vasodilator effect.[15] A direct way of stimulating Ang-(1–7) can be achieved by using Ang-(1–7) agonists,[67] because Ang-(1–7) levels are reduced in COVID-19 patients due to ACE2 downregulation.[66,67] However, Ang-(1–7) level in the arterial circulation of severe COVID-19 patients has been pointed out to be elevated in a 19-patients cohort, despite a decrease in Ang II levels.[69] Curiously, in null ACE2 mice, Ang II infusion increased Ang-(1–7) levels in lung cells, which suggests an ACE2-independent conversion pathway.[70] Therefore, a larger cohort is needed to deeper analyze the effects of ACE2 and Ang-(1–7) level in SARS-CoV-2 patients, and the administration of Ang-(1–7) agonists in the context of COVID-19 should be done carefully. The indirect way of stimulating molecules activated by ACE2 is providing exogenous ACE2 to the body, for instance, recombinant ACE2, previously suggested to protect mice from ARDS as the molecule reduced Ang II level in those animals lungs.[71]

It is worthy of mention that Ang-(1–7) agonists were initially preferred to restore ACE/ACE2 balance compared to Ang II blockers,[62,67] for the latter could increase ACE2 expression,[67] probably in a compensatory effect, and therefore could aggravate SARS-CoV-2 infection.[72] The administration of ACE inhibitors was uncertain for the same reasons.[72] However, there was not a difference in mortality comprising COVID-19-patients treated or not with ACE inhibitors and Ang II blockers in a 187-COVID-19-patients cohort.[72] Moreover, in an 8.3 million-people-cohort, among which there were 19,486 COVID-19-patients, ACE inhibitors and Ang II blockers were associated with reduced COVID-19 risks and were not associated with elevated risks of receiving intensive care.[73]

The fourth way is to block SARS-CoV-2 entry, so that the viral particle binds to an exogenous biomolecule instead of binding to transmembrane ACE2.[49,66] This can be conducted by using ACE2 peptidomimetics,[74] soluble ACE2,[49] by "tricking" SARS-CoV2 S protein by developing a nonactive mutated construct composed by the ectodomain of ACE2 fused to the Fc segment of immunoglobulin G-1 (IgG1),[75] by administering monoclonal antibodies,[76,77] and by using ensovibep,[78] which is a designed Ankyrin repeat protein (DARPin).[79] Peptidomimetics are modified peptides to better suit pharmacological properties and molecule stability,[80] in this case resembling

ACE2 and binding to SARS-CoV-2 S protein, which intends to prevent the viral entry.[74] Soluble ACE2 can reduce Ang II and also block viral entry by interacting with the S protein, preventing it from interacting with transmembrane ACE2.[66] Nonetheless, the use of soluble ACE2 shall be done carefully due to the greater amount of available circulating ACE2 in the body that may increase the number of viral particles attached to the enzyme, causing a prominent reduction in ACE2 expression by endocytosis in host cell.[66] Consequently, Ang II levels would increase as well, compromising blood pressure regulation, and aggravating disease severity.[66] In the construct composed by the ectodomain of ACE2 fused to the Fc segment of IgG1, the Fc segment can provide immune recognition and the maintenance of the ectodomain of ACE2 in the construct allows it to be recognized by the viral S protein.[75] However, as the ectodomain is mutated, ACE2 does not have enzymatic activity towards Ang II, which, therefore, would not be affected by the use of the construct.[75] Monoclonal antibodies, such as the human neutralizing monoclonal antibodies, for example, B38 and H4, can bind to the RBD from SARS-CoV-2 S protein, aiming different epitopes each one, and inhibit the interaction with ACE2.[76] Besides, viral titers were reduced in mice lung cells, revealing the potential of antibody-based therapeutics to treat SARS-CoV-2 infection.[76] In addition, on December 16th, 2021, the Food and Drug Administration (FDA) issued an emergency use authorization (EUA) for other monoclonal antibodies, namely bamlanivimab plus etesevimab (which are neutralizing monoclonal antibodies that bind to overlapping epitopes in SARS-CoV-2 RBD and that can be used when the viral strain is neither Gamma nor Beta ones, and the dealt strain must be susceptible to these antibodies),[77] casirivimab plus imdevimab (which are recombinant human monoclonal antibodies that bind to nonoverlapping epitopes in SARS-CoV-2 RBD),[77] and sotrovimab (which is a repurposed drug originally used to treat SARS-CoV and that binds to a conserved epitope in SARS-CoV and SARS-CoV-2).[77] DARPins are artificial proteins with repeat domains.[81] Structurally based on Ankyrin repeat proteins,[81] DARPins constructs are composed of a β-turn and two antiparallel α-helices.[81] DARPins act as binding proteins[81] and they can be used to target intracellular proteins and inhibit them[82]; as well as differentiate between the phosphorylated and nonphosphorylated states of the same posttranslationally modified protein.[83] In addition, the use of DARPins can be an alternative to monoclonal antibodies in targeted therapy,[81] for DARPins are smaller molecules, have lower tendency to aggregate and present more stability in different temperatures and pH.[81] Moreover, DARPins can be used as basis for more complex constructs.[84] This is achieved by the fusion between DARPins and other proteins, which generates multivalent and multispecific binding proteins.[84] Hence, DARPin-based drugs can benefit from either monovalent or multivalent constructs.[85] Ensovibep is a 85 kDa[78] multispecific DARPin[79] that has two human serum albumin-binding domains, called H1 and H2, to extend the systemic half-life of the construct; and three RBD-binding domains, namely R1, R2 and R3, at the C-terminal.[78] Ensovibep acts by its three RBD-binding domains targeting the RBD from SARS-CoV-2 trimeric S protein at the same time.[78] This leads the S protein to a locked open-conformation and to the occlusion of the ACE2-binding site.[78] The drug was tested in vitro[78] and in vivo,[78] and the respective results were the neutralization of the variants Alpha, Beta, Gamma, Delta, Delta Plus, Lambda, and Omicron; and an optimal dose of 10 mg/kg to treat Roborovski dwarf hamsters. Moreover, regarding the administration of ensovibep 24 h after SARS-CoV-2 infection, the animals showed improved condition at 2 days postinfection.[78] The observed results encouraged the analysis of ensovibep in clinical trials[79,86] as a potential new drug.[78] Ensovibep was on phase 2 clinical trial since April 1st, 2021 so that the efficacy of the molecule and its optimal dose could be established to treat ambulatory COVID-19-patients.[86] As a result, ensovibep, administered as single-dose-intravenous infusion, was considered safe and it was well-tolerated at doses 75, 225, and 600 mg.[79] Then, it will follow phase 3 clinical trial with the chosen dose of 75 mg.[79] In this context, Novartis and Molecular Partners announced on January 10th, 2022 that they would seek that the FDA issues an EUA for ensovibep.[79]

The fifth way concerns administration of ACE2 inhibitors, such as dalbavancin, a lipoglycopeptide antibiotic[87]; enalaprilat, an ACE inhibitor[88]; ledipasvir[89] and paritaprevir,[89] two direct-acting antiviral drugs used to treat chronic hepatitis C; and cetilistat, an antipancreatic lipase used to treat obesity.[90] Dalbavancin is a drug approved by the FDA that binds to ACE2 and prevents it from interacting with SARS-CoV-2 S protein-based on in silico analysis.[87] Indeed, dalbavancin interacts with ACE2 through four amino acid residues, Glu329, Gln325, Gln42, and Asp38,

which are important to the binding of the S protein.[87] This binding inhibition was corroborated by the administration of dalbavancin in mice and rhesus macaque, in which viral replication was inhibited.[87] In addition, dalbavancin was shown to inhibit cathepsin L by approximately 40% in vitro.[87] Enalaprilat, also an FDA-approved drug, was seen in silico as having a high binding affinity of 1.5 nM for ACE2.[88] Enalaprilat can inhibit ACE, reducing inflammation and blood pressure, and has the potential to inhibit ACE2, possibly preventing SARS-CoV-2 entry,[88] which may restore ACE/ACE2 balance. Ledipasvir and paritaprevir bindings to ACE2 were studied in silico, revealing that these drugs interact with the enzyme with high binding energy −399.338 kJ/mol and −377.593 kJ/mol, respectively.[89] Such negative energy results indicate that the molecules have a strong affinity for the enzyme pocket.[90] Cetilistat was seen in silico as a potential drug to inhibit ACE2 due to its high binding energy (−8.70 kcal/mol), which suggests that a stable complex is formed between the enzyme and the drug.[90] It is noteworthy that, although dalbavancin,[87] enalaprilat,[88] ledipasvir,[89] paritaprevir,[89] and cetilistat[90] are in FDA-approved drugs list, safety and dosage in patients infected with SARS-CoV-2 should be analyzed before the drugs are administered.

It is interesting to highlight that the interaction of the S protein from SARS-CoV with ACE2 seems to not depend on the conformation of the latter.[91] In fact, the S protein from SARS-CoV can interact with ACE2 and enter HEK293T cells even in the presence of the ACE2 inhibitor MLN-4760.[91] This compound is known for inducing conformational changes in ACE2 structure.[91] Thus, it is necessary to study if this scenario applies for SARS-CoV-2 as well and, if it does, if the use of an ACE2 inhibitor will have an effect on the infection.

It is crucial to mention that, when administering a drug to elevate ACE2 level or the level of a molecule stimulated by ACE2, pancreatic clinical parameters, such as the enzymes produced by the organ, should be previously analyzed. The importance of this relies on the fact that, if ACE2 concentration would be high in the pancreas, but low in other organs, the administration of ACE2 may worsen the situation and lead to pancreatic injury.

3.1.2 | TMPRSS2 and Cathepsins B and L

3.1.2.3 | General aspects

The TMPRSS2 is expressed in different organs[92] as a type II membrane protein[93] with 70 kDa,[94] as seen in Table 1. The TMPRSS2 is a serine protease[95] that plays different roles in cell function, including ACE2 cleavage, as shown in Table 1.[94–96] Additionally, it has been reported that TMPRSS2 is highly expressed in prostate and colon carcinomas.[97] Structurally, TMPRSS2 has three functional domains and is able to bind to calcium ions (Table 1).[94,98]

The catB/L is a lysosomal ubiquitously expressed enzyme[99] as seen in Table 1. Moreover, as also shown in Table 1, they are cysteine proteases[49] that belong to a family of papain-like proteases.[25,100] Besides, cathepsin L is a 34 kDa-endopeptidase[99,101] and cathepsin B is a 38 kDa-endo and exopeptidase.[102] In addition, catB/L take part in protein degradation and processing, such as converting zymogens and hormones to their active forms,[103] as well as in epidermal and cardiac homeostasis, as presented in Table 1.[100] However, catB/L are also involved in pathological events, such as inflammatory respiratory disease, tumor invasion and metastasis, osteoporosis, and Alzheimer's Disease.[103] Structurally, the cathepsins have two domains and conserved catalytic sites (Table 1).[99]

3.1.2.4 | In SARS-CoV-2 infection

The TMPRSS2 and CatB/L play an important role in SARS-CoV-2 infection by processing the viral S protein after it binds to ACE2,[25] as seen in Figure 2 and Table 1. The SARS-CoV[91] and SARS-CoV-2[25] S proteins are responsible for the viral particle attachment to the host cells, through interaction with ACE2, and membrane fusion, respectively by the domains S1, which houses the RBD, and S2 from the viral proteins (Table 1).

Besides, there are two proteolytic cleavage sites regarding the viral S1 and S2 domains (Table 1). The first one lies between the two domains and is named S1/S2; the second one is located within the S2 domain and is named S2′.[25] After ACE2 binding, the S protein undergoes a conformational change and two proteolytic cleavages.

The first one occurs at the S1/S2 site and results in the release of S1 and S2. The second cleavage occurs at the S2′ site and leads to membrane fusion[25] and consequent entry of the virus into the cell by endocytosis.[49]

Indeed, it was shown that SARS-CoV-2 entry depends on the priming activity of CatB/L and TMPRSS2.[25] TMPRSS2 is responsible for cleavage of S1/S2 and S2 subunit[25] and both CatB/L cleave S1/S2 site.[104] Nonetheless, cathepsin B is approximately fourfold more active against SARS-CoV-2 than cathepsin L, and the opposite goes for SARS-CoV (approximately 10-fold more active).[104] Besides, TMPRSS2 is known for cleaving ACE2, which could increase SARS-CoV infectivity[96] and maybe of SARS-CoV-2 as well. Also, in SARS-CoV-2 infection, TMPRSS2 expression itself is increased due to interleukin-1β activity.[105]

However, TMPRSS2 is important, but not essential for SARS-CoV-2 entry and spread in the host,[25] because in the absence of TMPRSS2, SARS-CoV-2 uses CatB/L to enter the cell.[25] Thus, the use by SARS-CoV-2 of two host proteases to allow its entry into the cell is a survival strategy.[21] Indeed, in lung and primary human airway epithelial cells, TMPRSS2 is used by SARS-CoV-2 as an entry route that is independent from endosome.[21] By avoiding endosome, SARS-CoV-2 also escapes interferon inducible transmembrane (IFITM) proteins,[21] which is a protein family located in the endosome/lysosome that targets viruses that use those cell pathways for transport inside the cell.[106]

Moreover, since the proteolytic cleavage of the SARS-CoV-2 S protein is an essential step during infection, it can be a therapeutic target of interest,[25] as seen in Table 1. Camostat mesylate is an example of TMPRSS2 inhibitor.[25] In silico studies have pointed out that paritaprevir, daclatasvir, and ombitasvir, previously used against hepatitis C virus, can inhibit TMPRSS2 with high binding affinities, respectively 8.75, 6.66, and 5.91 nM.[88] In addition, eprosartan, an inhibitor of Ang II receptor, and lisuride, a neuropsychiatric drug, were also seen in silico as potential TMPRSS2 inhibitors, with respective high binding affinities of 9.19 nM and 11.20 nM.[88] However, clinical data about the use of TMPRSS2 inhibitors against SARS-CoV-2 are currently lacking in the literature.[107] For the diseases caused by CatB/L, as seen in Table 1, both have become potential therapeutic targets[103] for synthetic drugs[108] and natural products,[109] especially those with reversible and noncovalent binding.[103] An example of cathepsin B[110] and L[111] inhibitor is E-64, an epoxy succinyl-based molecule, which has irreversible activity.[112] Indeed, it has already been shown that E-64 can inhibit cathepsin L in MDA-MB-231 cells, a human breast cancer lineage, but despite E-64 selectivity, off-target inhibition may also happen because it needs to access the intracellular environment. This may occur for cathepsin L, which is located in the cytoplasm,[111] but could also happen for cathepsin B, since it is found in the same cellular environment.[112] Indeed, it is yet to be determined if these inhibitors, which act on TMPRSS2 and CatB/L, are really good choices to treat SARS-CoV-2 infection, as well as the effects they may cause in COVID-19 patients.

3.1.3 | The JAK/STAT pathway

3.1.3.5 | *General aspects*

The Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) signaling pathway is ubiquitously expressed in the human body.[113] It is responsible for transmitting information from the extracellular to the intracellular environments.[114] Indeed, the JAK/STAT pathway is a tyrosine kinase cascade,[24] which is activated in the course of a viral infection.[11] The presence of viral particles in the body releases type I interferon (IFN),[24] which comprises IFN-α and IFN-β.[11] Type I IFN interacts with IFNα/β receptor (IFNAR)[11] and this initiates the transcription of IFN-stimulated genes (ISGs).[24,114] The ISGs act on the upregulation of antiviral proteins, the release of cytokines and recruitment of immune cells.[115]

3.1.3.6 | *In SARS-CoV-2 infection*

It is expected that SARS-CoV-2 follows the model of infection of SARS-CoV,[24] which inhibits STAT-1,[11] and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), which in the late phase of infection is known for flaring the

cytokine storm.[29] Indeed, proteomic analysis has shown that SARS-CoV-2 targets different host kinases, among which there is the tyrosine-protein kinase (KIT), through the viral protein open reading frame 3a (ORF 3a).[36] Besides, KIT can phosphorylate, and thus activate, JAK-3, STAT-1, -3 and -5 in HMC-1 cells, a lineage of mast cell leukemia.[116] Therefore, administration of type I IFN would result in exacerbating the immune response to the virus by recruiting neutrophils, monocytes, and macrophages, stimulating cytokine response and inhibiting lymphocytes T response.[29] Figure 2 shows a simplified representation of the role played by JAK/STAT in SARS-CoV-2 infection.

Besides, it has been suggested that anti-inflammatory drugs that inhibit the JAK/STAT signaling pathway can be used to treat SARS-CoV-2 infection.[30] Among the drugs already reported in other papers, baricitinib,[30] fedratinib,[30] and ruxolitinib are highlighted.[30] Baricitinib, an inhibitor of JAK-1 and JAK-2,[117] seems to be the most suitable drug for this purpose due to its properties, such as low plasma protein binding, minimal interaction with cytochrome P450 (CYP) enzymes and drug transporters, and therapeutic doses. In addition, baricitinib could be combined with antivirals, for example, ritonavir and remdesivir, to reduce viral infectiveness, viral replication, and the inflammatory responses during SARS-CoV-2 infection.[30] However, the inhibition of JAK-1 and JAK-2 by baricitinib could negatively affect the interferon-mediated antiviral response, which could lead to the opposite effect and facilitate the course of SARS-CoV-2 infection, favoring other pathogens to invade the body.[118] Moreover, baricitinib was used to treat a male COVID-19-patient with as an oral dose of 4 mg/day/2 weeks,[119] but at this concentration it can cause thromboembolism[120]; thus, efficacy and safety studies are still needed before it can be added to the COVID-19 protocol treatments.

3.1.4 | Other human enzymes that are involved in SARS-CoV-2 infection

3.1.4.7 | Cullin ligase

The Cullin ligase is the center of a protein complex, acts as an E3 ligase, and is responsible for transferring ubiquitin to its substrates.[121] Proteomic analysis has revealed that the subunit ZYG11B of Cullin ligase seems to be targeted by viral ORF10 and, ironically, be driven to ubiquitination and degradation, as well as ZYG11B may drive ORF10 to degradation.[31]

3.1.4.8 | Ubiquitin specific peptidase 13 (USP13)

The USP13 is another component of the ubiquitin-proteasome pathway, but, although able to interact with SARS-CoV-2, the details are still missing. It is known, however, that its role in different cancer types depends on the context and that it may influence the infiltration of immune cells in some tumor types.[32]

3.1.4.9 | Deaminases APOBEC and ADAR

3.1.4.9.1 | *General aspects.* The ADARs are adenosine deaminases, which act as adenosine (A) to inosine (I) RNA editing enzymes by deaminating double-stranded RNA,[122] which are commonly associated with exogenous nucleic acid and are able to induce IFN responses.[123] Interestingly, ADAR1 can downregulate IFN-inducible transcripts, regulating this cytokine activity and thus protecting organisms from chronic inflammation. However, ADAR1 may have a proviral activity, both in the presence and in the absence of RNA editing activity.[124] Besides, in humans, APOBEC3s are cytidine deaminases,[125] which act as a cytosine (C) to uracil (U) RNA editing enzyme,[126] but some members of this family can also mutate C to U in DNA,[127] by deaminating both single-stranded RNA and DNA.[33]

3.1.4.9.2 | *In SARS-CoV-2 infection.* The APOBEC3s have antiviral activity, taking part in innate and adaptive immune responses, regarding different viruses, such as retroviruses, parvoviruses, herpesviruses and hepatitis B virus.[128] However, viruses have evolved to evade APOBECs,[128] such as human coronavirus (HCoV)-NL63, which is

inhibited by APOBEC3s A3C, A3F, and A3H.[129] Interestingly, the APOBEC3 effect against the virus was mostly due to its deaminase activity, for although lack of the enzymatic activity reduced APOBEC inhibition power, the difference in infectivity rate was not significant between controls and cells with mutated versions of those APOBEC3s.[129] This antiviral activity without deaminase activity may be due to the fact that APOBEC3s A3C, A3F, and A3H still interact with HCoV-NL63 through the viral N protein, which be corroborated by the lack of observed hypermutations.[129] Nonetheless, it is not conclusive if APOBEC3s do play a role in editing the SARS-CoV-2 RNA genome. The MERS-CoV, SARS-CoV, and SARS-CoV-2 seem to not have the APOBEC3 footprint NTC, seen in HCoV-229E, HCoV-OC43, and HCoV-HKU1.[130] The APOBEC3 footprint is the under-representation of its target motifs, and NTC is a nucleotide sequence in which the C is the third position of the codon.[130] Then, not having the APOBEC3 footprint NTC means that MERS-CoV, SARS-CoV, and SARS-CoV-2 can hide from APOBEC3s.[130] Nonetheless, ADARs and APOBEC3s have been suggested as the resources of A to I and C to U editing observed in SARS-CoV-2 RNA genome.[33] However, these hypotheses still need to be analyzed in the future.[33] In case of confirmation that APOBEC3s do play a role in editing the SARS-CoV-2 genome, it would be essential to study polymorphisms in APOBEC3A and APOBEC3B, which are common in Chinese people, in the context of COVID-19.[33]

3.1.4.10 | *The SERPINs*

3.1.4.10.3 | *General aspects.* The SERPINs are a protein superfamily of mostly serine proteases, but some of them also have cysteine protease activity,[38] which means that they can inhibit serine proteases or cysteine proteases, respectively.[131] Indeed, the SERPIN antithrombin, which inhibits serine proteases, is able to inhibit papain and cathepsin L, which are cysteine proteases,[132] and the SERPIN α-antitrypsin, which inhibits serine proteases, mutated to inhibit cysteine proteases has been shown to inhibit cathepsins L, V and K, but not papain or cathepsin B.[133]

SERPINs are phylogenetically grouped in 16 clades, from A to P,[134] and the nomenclature is followed by a number within each clade.[131] However, SERPINs can also be known by alternative names.[131] Interestingly, in vertebrates, SERPINs can group with others from different functions,[134] for example, in clade A there are inflammatory response molecules, such as SERPINA1 (antitrypsin) and SERPINA3 (antichymotrypsin), and noninhibitory hormone-transport molecules, as SERPINA6 (corticosteroid-binding globulin) and SERPINA7 (thyroxine-binding globulin).[131]

Structurally, SERPINs have a single core domain, composed of three β-sheets and 8 or 9 α-helices.[37] The reactive center loop (RCL) of SERPINs is above the scaffold of the molecule and it is the region responsible for the interaction with the targeted proteases.[131] Besides, SERPINs differ in their glycosylation profile, which results in a range of molecular weights from 40 to 100 kDa.[135] SERPINs are ubiquitously expressed in the human body, although a higher expression is seen in the liver.[135]

As briefly mentioned above, SERPINs can act in either two different ways, an inhibitory one, such as SERPINA1 and SERPINA3, or a noninhibitory one, for example, SERPINA6 and SERPINA7,[131] as a result of the five conformational states of SERPINs, namely native, cleaved, latent, δ, and polymeric, the main difference among them being the structure of the RCL.[134] The native conformation of SERPINs is characterized by the exposition of the RCL and its availability to bind to proteases.[134] The native state of SERPINS is metastable, which means that it has higher free energy than the most stable state.[136] The cleaved form results after the SERPINs structures are cleaved by the target protease, and it is recognized by the movement of RCL into the β-sheet A of the SERPINs themselves,[137] which results in a more stable conformation, also known as relaxed state.[134] The latent structures are alternative relaxed states, in which the RCL is also inserted into the β-sheet A, but there is no previous cleavage.[134] The latent states are noninhibitory conformations[134] that can act as a control mechanism.[131] The δ-form is intermediate to the native and latent forms.[134] The polymeric conformation is known by the RCL of a

SERPIN docking into the β-sheet A of another SERPIN molecule, generating an inactive SERPIN polymer that can lead the body to serpinopathy.[131]

When it comes to the inhibitory action, the RCL is important to stabilize the inhibition, but it is not necessary for the protease to bind the SERPINs.[138] Hence, the RCL undergoes conformational changes during the inhibitory path that affect the whole fold of the SERPINs,[134] in which the serine from the active site of the target protease interacts with the RCL from SERPINs.[139] Then, similarly to a protease-substrate interaction, the P1-P1′ peptide bond of the RCL is cleaved by the target protease and a covalent acyl-intermediate is formed.[136] The cleaved RCL is inserted into β-sheet A,[136] which is a thermodynamically more stable conformation of SERPINs.[137] Thus, the movement of the RCL from SERPINs induces a change in the conformation of the protease too, to the point of distortion of the enzyme[136] and a loss of 37% of structure in the case of the protease trypsin, for example.[139] Truly, in the case of trypsin, the catalytic serine (Ser195) is moved away from the histidine (His57) of the catalytic site, impairing the whole protease structure, even the calcium-binding site, and it inhibits the release of trypsin from the complex SERPIN-protease.[139] Therefore, the inhibitory SERPINs can "trap" proteases in a complex in the acyl-intermediate form.[136] Besides, in the case of chymotrypsin, the complexation with antichymotrypsin leads the protease to proteolysis.[140] Comprising their roles in the human body, through their inhibitory activity, SERPINs act as regulators of fibrinolysis, immune responses, and inflammation.[135]

The noninhibitory action may be due to a loss of the inhibitory function so that SERPINs could play other more specialized roles.[141] Truly, SERPINs act as regulators of hormone binding and hormone precursors through their noninhibitory activity.[135] Indeed, angiotensinogen, the precursor of angiotensin I, has the SERPIN motif [142] at the C-terminal,[143] and the molecule is also known as SERPINA8.[144] Also, the noninhibitory path can be a result of inter- or intramolecular chemical modifications, such as oxidation, polymerization (inter- or intramolecular ones), complex formation and cleavage by nonspecific proteases, as already seen for α1-antitrypsin,[145] also known as SERPINA1.[131] Indeed, the complexation of SERPINs with proteases may have a role in the inflammation process.[145] For example, α1-antitrypsin is responsible for inhibiting overexpressed proteases during inflammation, and therefore α1-antitrypsin can regulate eventual tissue damage.[145] This regulation occurs when, during inflammation, α1-antitrypsin present in tissues complexes with neutrophil elastase, this leads neutrophils, monocytes and alveolar macrophages to increase the expression of α1-antitrypsin and its plasma concentration is increased.[145] In addition, it is worthy to mention that exacerbated proteolytic activities contribute to respiratory diseases, for instance, asthma, cystic fibrosis, chronic obstructive pulmonary disease, acute lung injury and ARDS.[146]

Moreover, SERPINs acting as a "trap" to target proteases[135] and inhibiting them[34] suggests that SERPINs may be used as treatment for pathologies caused by enzymes.[135] Besides, SERPINs play some roles in infections, for instance, by inhibiting pathogenproteases and pathogen binding, by preventing host apoptosis caused by pathogen invasion, and by enhancing host immune system.[137] A case that should be noted is the one of α1-antitrypsin treatment preventing neutrophil elastase from degrading the short palate, lung, and nasal epithelium clone 1 (SPLUNC1) protein, as shown by Jiang et al.[147] SPLUNC1 is produced in the large airway epithelial cells and it is responsible for host defense against pathogens, such as *Pseudomonas aeruginosa*.[147] However, *P. aeruginosa* unleashes SPLUNC1 degradation by neutrophil elastase. α1-antitrypsin can inhibit neutrophil elastase in the lungs, contributing to defend the organ from pathogens.[147] In this context, wild type mice infected with *P. aeruginosa* and then treated with exogenous α1-antitrypsin were seen to have, in their lungs, a reduced *bacterium* load and an increase in SPLUNC1 level.[147]

3.1.4.10.4 | *In SARS-CoV-2 infection.* It has been shown by proteomic analysis that SERPINA5 takes part in hemostasis and acts as an inflammatory factor, and can be reduced in COVID-19 patients.[36] In addition, SERPINA3 levels seems to be increased in the disease.[35] Interestingly, recombinant SERPINs with cysteine protease activity have been suggested as a trap to SARS-CoV-2 M^pro, since the viral enzyme is a cysteine protease,[148] but the success of this idea still needs to be investigated. It has been shown that SERPINA1 is able to inhibit TMPRSS2 in a

dose-dependent way.[34] The inhibition of TMPRSS2, a serine protease,[95] could inhibit or at least limit SARS-CoV-2 infection, as well as the alveolar inflammatory response, since SERPINA1 is delivered to the lungs and also inhibits the neutrophil elastase and therefore innate immune mediators. Besides, SERPINA1 is already approved by the FDA to treat α1- antitrypsin (A1AT) deficiency,[34] but clinical studies regarding its administration in COVID-19 patients are still missing.

3.2 | Viral enzymes

3.2.1 | General aspects

Coronaviruses have two proteases, M[pro] and PL[pro],[9] respectively known as nsp5 and nsp3.[10] The SARS-CoV proteases are fundamental to the viral replication cycle (Table 2) by processing their own polyproteins, after the virus enters the cell and the genomic RNA is translated into polyproteins (pp) 1a and 1ab. Indeed, M[pro] and PL[pro] release themselves from pp1a and pp1ab by autoproteolytic activity,[149] cleaving the polyproteins in different regions: M[pro] processes the central[149] and the C-terminal region of pp1a/pp1ab, while PL[pro] cleaves the N-terminal region (Table 2).[9]

In addition, viruses can modulate the host ubiquitin system,[150] by deubiquitylase activity,[151] can modulate the host ubiquitin system,[150] so that the success of the infection can be achieved.[152] Viral invasion may also induce the expression of ISG-15 in the host body,[153] a 15 kDa ubiquitin-like protein,[154] which is a product of the type I IFN signaling pathway that aims, among other targets, JAK and STAT proteins,[155] and can be targeted by viral deISGylases (enzymes that cleave ISG[151] and reverse its signaling[153]) as a survival strategy.[150,152,153,155,156] Coronaviruses PL[pro], for example, also has deubiquitylase and deISGylase activities,[27] as seen in Table 2.

3.2.2 | The main protease (M[pro])

Such as SARS-CoV M[pro],[9] the SARS-CoV-2 M[pro] is a cysteine protease with a cysteine-histidine catalytic dyad, as seen in Table 2.[26] The M[pro] is also called 3C-like protease (3CL[pro]) due to its structural similarity to picornavirus 3C proteases.[149] Indeed, SARS-CoV-2 M[pro] is a chymotrypsin-like protease[156] containing three structurally different domains (Table 2).[26,156]

The inhibition of SARS-CoV-2 M[pro] seems to be an attractive therapeutic strategy against COVID-19 due to its fundamental importance for the virus replication cycle (Table 2).[10,156] Besides, there is no human protease with a similar recognition sequence for cleavage as viral M[pro] does. Thus, SARS-CoV-2 M[pro] inhibitors are supposed to be nontoxic,[156] or at least to have lower toxicity.

Suggested pharmacological approaches include, as shown Table 2, Michael acceptors with a peptidyl region specific for proteases[26] and repurposing of commercially available drugs.[26,135] Among repurposed drugs there are ebselen,[26] cinanserin,[26] PX-12,[26] and pyridone-containing[156] inhibitors. Ebselen is an organoselenium compound with anti-inflammatory, antioxidant and cytoprotective activities.[26] Cinanserin is a serotonin antagonist, also known for inhibiting SARS-CoV M[pro].[26] PX-12 is an apoptosis stimulator, down regulator of vascular endothelial growth factor, and tumor inhibitor.[26] Pyridone-containing inhibitors[156] are natural products that have antimicrobial, -viral and -inflammatory properties.[157] However, these drugs still need to undergo clinical trials to evaluate their efficacy and safety before they take part in the SARS-CoV-2 infection treatment protocol.

Pfizer Inc. developed Paxlovid (PF-07321332/ritonavir),[158] which is composed of nirmatrelvir,[159] an M[pro] inhibitor, coadministered with a low dose of ritonavir.[158] The FDA issued an EUA for the drug[159] after phases 2/3 clinical trial were conducted for evaluation of safety and efficacy in adult patients.[158] Among the advantages of Paxlovid, its oral administration and specificity to target M[pro] are highlighted.[158] As an oral drug, Paxlovid can be

TABLE 2   Viral enzymes involved in SARS-CoV-2 infection that can be useful drug targets

Features	Viral Proteases	
	The main protease (Mpro) or 3C-like Protease (3CLpro)	The papain-like Protease (PLpro)
Location in the Viral Particle	This protease is produced after host cells invasion, Its coding sequence is in the nonstructural protein (nsp)-5	This protease is produced after host cells invasion, Its coding sequence is in the nonstructural protein (nsp)-3
Enzymatic Function	C-proximal cysteine protease (LQSAG)	N-proximal protease (LXGG) Deubiquitination (LRGG) DeISGylase (LRGG) Zinc-binding (CTC)
Structure	Homodimer, Chymotrypsin-like protease with three domains, Between domains I and II belongs the catalytic site, Domain III houses the C-terminal region, Catalytic site is a dyad of cysteine and histidine, Domain III is required for homodimerization, Domain I and II have an antiparallel beta-barrel globular cluster, Domains II and III are connected by a loop region, Domain III is a dimerization regulator, The region named N-finger comprises an interface region, composed of domain II of one protomer and the N-terminal residue of the other The catalytic site is formed by interaction between the N-finger of each of the two protomers, with squeezing between domains II and III of its own monomer and domain II of the other monomer Connecting both catalytic sites there is a channel-shape region	Monomer, This viral protease has four domains similarly to SARS-CoV: thumb, palm and fingers domains compose the right-hand architecture; and at the N-terminal there is an ubiquitin-like domain, Between thumb and palm domains relies the catalytic site, Catalytic site is a triad composed of cysteine-histidine-aspartic acid, The fingers domain houses a cysteine residue that coordinates a zinc ion, To SARS-CoV, the zinc ion is fundamental to the PLpro conformation and activity
Role in SARS-CoV-2 Infection	After cell invasion and synthesis of the viral polyproteins (pp) pp1a (~450 kDa) and pp1ab (~750 kDa), which are overlapped, this protease releases itself from them both by autoproteolytic activity and processes the central and the C-proximal region of pp1a/pp1ab	After cell invasion and synthesis of the viral polyproteins (pp) pp1a (~450 kDa) and pp1ab (~750 kDa), which are overlapped, this protease releases itself from them both by autoproteolytic activity and processes the N-proximal region of pp1a/pp1ab, The deubiquitination and deISGylase help the virus evade the host immune system
Biotechnological Approach	This protease is of fundamental importance in the virus replication cycle and there is no human protease with a similar recognition sequence for cleavage,	This protease is of fundamental importance in the virus replication cycle; Suggested pharmacological approaches include inhibitors of SARS-CoV PLpro; repurposed drugs, such as the ones that

(Continues)

TABLE 2 (Continued)

Features	Viral Proteases	
	The main protease (M^pro) or 3C-like Protease (3CL^pro)	The papain-like Protease (PL^pro)
	which, theoretically, makes SARS-CoV-2 M^pro inhibitors nontoxic molecules, Suggested pharmacological approaches include Michael acceptors with a peptidyl region specific for proteases; repurposing of commercial available drugs, such as ebselen, cinanserin and PX-12; and pyridone-containing inhibitors; the FDA issued an EUA on Paxlovid from Pfizer in coadministration with ritonavir	act via S3/S4 pockets, ticlopidine (inhibitor of platelet aggregation), procainamide (antiarrhythmic), labetalol (antihypertensive), amitriptyline (antidepressant), formoterol (antiasthma); GRL0617 (previously known as a SARS-CoV PL^pro inhibitor), YM155 (an anticancer drug candidate). Inhibiting this enzyme needs special attention due to a possible similarity with host deubiquitinating enzymes

Abbreviations: 3CL^pro, 3C-like protease; EUA, emergency use authorization; FDA, Food and Drug Administration; M^pro, main protease; nsp, nonstructural protein; PL^pro, papain-like protease; pp1a, polyprotein 1a; pp1ab, polyprotein 1ab; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2.

easily administered.[158] As an M^pro inhibitor, the drug is supposed to have lower toxicity, as previously discussed. Indeed, regarding 1881 patients, 1.7% of them presented serious adverse events versus 6.6% patients dosed with placebo.[158] The coadministration with ritonavir intends to slow the metabolism of the drug, allowing it to be active for a longer time in the body.[158] Paxlovid data is under review by the European Medicines Agency (EMA) since November 19th, 2021 to provide recommendations to countries from the European Union that want to include the drug in their protocols against COVID-19 in the current trial step.[160] Besides, on January 10th, 2022, EMA started evaluating an application for the commercialization of Paxlovid to treat COVID-19 patients that are 12 years old or older (weighing at least 40 kg) with mild to moderate infection who are at risk of progressing to severe symptoms.[161] However, it is worth to point out that the administration of Paxlovid to uncontrolled or undiagnosed human immunodeficiency virus (HIV)-1-patients may cause HIV-1 drug resistance.[159] Ritonavir can cause liver damage, therefore, patients with liver diseases or liver inflammation must be carefully monitored.[159] Severe kidney impairment is also a cause to not use Paxlovid.[159]

3.2.3 | The papain-like protease (PL^pro)

Interestingly, SARS-CoV has only one PL^pro, whilst other coronaviruses have two of them, which could be a result of the evolution process of the lineage.[162] The SARS-CoV PL^pro, different from M^pro, presents a catalytic triad, as shown in Table 2. In addition, it requires stabilization of negative charges during peptide hydrolysis, which is provided by the formation of an oxyanion hole.[163]

Furthermore, SARS-CoV-2 PL^pro recognizes the LXGG motif found in viral nsp and the LRGG motif present at the C-terminal of ISG15 and ubiquitin.[27] Nonetheless, SARS-CoV-2 PL^pro is about 10 times more active in its deISGylase function than in its deubiquitination, and the former may be even higher than its classical proteolytic activity,[28] which may contribute to evasion from the innate immune response.[27] Indeed, the deISGylase activity is conserved not only among coronaviruses, but in influenza B as well, pointing out the importance of this activity.[164]

Interestingly, SARS-CoV and SARS-CoV-2 PL^pro share 83% of identity and 17% of similarity regarding the first ubiquitin-like site, and 67% of identity and 13% of similarity regarding the second one.[27] Moreover, SARS-CoV-2 PL^pro has four domains similar to SARS-CoV PL^pro: thumb, palm and finger domains, which compose the right-hand architecture, and an ubiquitin-like domain at the N-terminal. The catalytic site lies between the thumb and palm

domains.[28,163] Both in SARS-CoV[163] and SARS-CoV-2 PL^{pro},[28] the finger domain houses a cysteine residue that coordinates a zinc ion. In SARS-CoV PL^{pro}, the zinc ion is critical to enzyme conformation and activity.[163]

However, regarding activities, SARS-CoV-2 PL^{pro} resembles MERS-CoV and not SARS-CoV PL^{pro}.[28] Indeed, it has already been hypothesized that MERS-CoV PL^{pro} may recognize and process ubiquitin and ISG15 differently than SARS-CoV PL^{pro}. This is due to the fact that MERS-CoV and SARS-CoV share little sequence conservation in the ridge region.[165]

Given the role played by PL^{pro}, the structural similarity between the PL^{pro} from SARS-CoV and the one from SARS-CoV-2, and their profile of recognized substrates, it has been suggested that inhibitors of SARS-CoV PL^{pro} may be used against SARS-CoV-2 PL^{pro}, as well (Table 2).[27] Furthermore, regarding COVID-19, the repurposing of some drugs available to treat other diseases has been suggested. Some of those might act against SARS-CoV-2 PL^{pro} via S3/S4 pockets, which, although more distant from the catalytic site, are larger than the S1/S2 pockets. Among the suggested repurposed drugs that bind in silico to SARS-CoV-2 PL^{pro} are ticlopidine (inhibitor of platelet aggregation; binding affinity for PL^{pro} of 160 nM–16 µM), procainamide (antiarrhythmic; binding affinity for PL^{pro} of 30 nM–3 µM), labetalol (antihypertensive; binding affinity for PL^{pro} of 113 nM–11 µM), amitriptyline (antidepressant; binding affinity for PL^{pro} of 466 nM–46 µM), and formoterol (antiasthma; binding affinity for PL^{pro} of 716 nM–71 µM).[166] Besides, GRL0617, a naphthalene-based compound previously known as a SARS-CoV PL^{pro} inhibitor, showed in vitro IC$_{50}$ of approximately 2.1 µM for SARS-CoV-2 PL^{pro}.[167] GRL0617 acts mainly on the enzyme deISGylating activity through a noncovalent interaction rather than on the deubiquitinating activity in infected HEK293T cells.[167] Another compound, YM155, an anticancer drug candidate, was shown to target SARS-CoV-2 PL^{pro} in the substrate-binding pocket, the ISG15-binding site, and the zinc finger motif.[168] The IC$_{50}$ for YM155 was 2.47 µM, against the IC$_{50}$ of 1.39 µM seen for GRL0617 in the same paper.[168] However, the EC$_{50}$ value was higher for YM155 (0.17 µM) than for GRL0617 (3.18 µM), which suggests that YM155 has a more potent antiviral activity than GRL0617.[168]

In addition, SARS-CoV PL^{pro} shows a certain degree of homology with human deubiquitinating enzymes,[169] and SARS-CoV and SARS-CoV-2 PL^{pro} are quite similar.[27] Thus, there is a need to establish if SARS-CoV-2 PL^{pro} is homologous to human deubiquitinating enzymes as well. If so, drug candidates against SARS-CoV-2 PL^{pro}, as well as against SARS-CoV PL^{pro}, must not inhibit human deubiquitinases. Finally, the in vivo potency of inhibitors still needs to be confirmed and optimized for their use as therapeutics agents.[169]

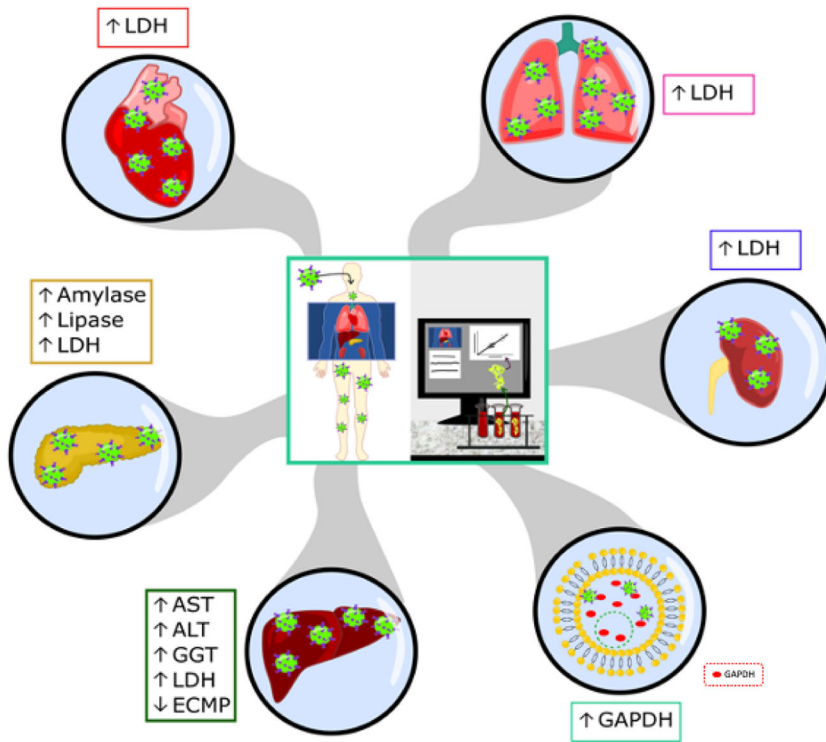# 4 | ENZYMES CAN BE PROGNOSIS BIOMARKERS IN SARS-COV-2 INFECTION

It has been reported that the levels of certain enzymes can be altered in blood samples from patients infected with SARS-CoV-2 (Figure 4). These enzymatic tests can be useful since, despite the symptoms displayed by the patients, physical examination of cardiovascular, abdominal and neurological profiles do not show alteration.[6] Among the enzymes whose levels are altered upon SARS-CoV-2 infection. There are amylase and lipase,[54,170] cholesterol metabolism pathway[171]; aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (GGT)[6]; lactate dehydrogenase (LDH)[172]; and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).[171]

4.1 | Amylase, lipase, and enzymes from the cholesterol metabolism pathway

4.1.1 | General aspects

Amylase and lipase are common biochemical markers of acute pancreatitis since both enzymes are produced by pancreatic acinar cells.[173] Besides, in the human body, the cholesterol synthesis comprises over 15 enzymes,

FIGURE 4 Profile of the altered enzymes that can be used as prognosis biomarkers in COVID-19 and the main organs that are compromised by SARS-CoV-2. Enzymes whose levels are elevated in SARS-CoV-2 infection: LDH: lactate dehydrogenase (intracellular enzyme produced in the liver, pancreas, kidneys, and heart and responsible for converting pyruvate into lactate); AST: aspartate aminotransferase (cytoplasmic and mitochondrial enzyme present in hepatocytes); ALT: alanine aminotransferase (cytoplasmic enzyme present in hepatocytes); GGT: γ-glutamyltransferase (membrane enzyme in hepatocytes); GAPDH: glyceraldehyde 3-phosphate dehydrogenase (cytoplasmic ubiquitous enzyme from the glycolytic pathway); amylase and lipase: common biochemical markers of acute pancreatitis because they are produced by pancreatic acinar cells. Enzymes whose levels are reduced in SARS-CoV-2 infection: ECMP: enzymes from the cholesterol metabolism pathway (active in the liver). COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2 [Color figure can be viewed at wileyonlinelibrary.com]

such as 3-hydroxy-3-methylglutaryl-coenzyme A synthetase (HMGCS), and 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGCR), and its absorption stage includes pancreatic and hepatic lipases.[174]

Despite there is still no evidence that pancreatic damage is caused by SARS-CoV-2 and no viral nucleic acid was found in pancreas,[54] an increase in amylase and lipase levels has also been seen in COVID-19 patients and this profile has been associated with pancreatic injury,[170] sometimes as an acute pancreatitis.[175] Some possible explanations for this type of pancreatitis might be the viral cytopathic effect in pancreas, systemic responses to respiratory injury or even a response to the immune cascade developed to stop the viral infection.[170]

Therefore, it is possible that amylase and lipase levels are altered in SARS-CoV-2 infection as they are produced by the pancreas, and by salivary glands in the case of amylase, and cleared by the kidneys. However, whether the high levels of amylase and lipase in COVID-19 patients are due to pancreatitis needs to be confirmed on a large scale.[176]

4.1.2 | Amylase in SARS-CoV-2 infection

A case report regarding SARS-CoV-2 patients showed that amylase concentration in blood can increase (Figure 4) from 173 U/L (at hospital admission time) to >1500 U/L (11 h after hospital admission) and from 85 U/L (at hospital admission time) to 934 U/L (6 days after hospital admission), in both cases with a Modified Glasgow Acute Pancreatitis Score of five points.[175] The diagnosis of acute pancreatitis was also supported in these cases by imaging tests and reported abdominal pain.[175] Although it was not clear if the virus contributed to the pancreatic injury, the importance of measuring amylase levels in SARS-CoV-2 infection cases remains,[175] since viral infections can lead to acute pancreatitis.[173]

4.1.3 | Lipase and enzymes from the cholesterol metabolism pathway in SARS-CoV-2 infection

A multivariate analysis has shown that higher than basal lipase levels (>156 U/L, surpassing more than three times the upper limit of normal) also can be related to severe cases of COVID-19.[177] However, this analysis lacked abdominal imaging of the pancreas, thus it was not possible to establish the origin of lipase elevation, which can either be pancreas injury or multiorgan dysfunction, or even both.[177] In another analysis, lipase levels >180 U/L were reported in SARS-CoV-2 patients, and therefore, higher than 60 U/L, which is considered the normal limit level[178]. Interestingly, it has been pointed out that higher lipase levels are not restricted to pancreatitis and that a colonic or enteric SARS-CoV-2 participation might be the cause of elevated lipase levels.[178]

Besides, proteomic analysis has suggested that proteins from the cholesterol metabolism pathway can be negatively influenced by SARS-CoV-2 infection,[171] as seen in Figure 4. Interestingly, cholesterol itself in the tissue, not in serum, has been pointed out as a possible influencer of ACE2 and TMPRSS2 activities, as a ruler of the size and organization of lipid rafts so that enhancing cholesterol levels improves SARS-CoV-2 binding to the cell surface.[179] It is known, since studies with SARS-CoV, that lipid metabolism can be altered in committed patients even after 12 years of recovery.[180] It would be of interest in the future, to better understand the mechanisms of action of cholesterol in SARS-CoV-2 patients, to analyze and correlate cholesterol concentrations to the levels of lipase and other enzymes from the cholesterol pathway.

Curiously, although increasing ACE2 expression, it has been shown that administration of statins, for example, atorvastatin, rosuvastatin, simvastatin, pravastatin, fluvastatin and pitavastatin, in hospitalized SARS-CoV-2 patients can diminish the risk of mortality compared to the nonuse of statins. Therefore, they could be safely used alone or combined with ACE inhibitors or angiotensin II receptor blockers to treat COVID-19.[181] Nonetheless, it should be taken into account that administration of statins can cause myalgia, myopathies, and rhabdomyolysis, which can lead to acute kidney injury. Kidney diseases are already associated with SARS-CoV-2 infection and the risks can be reinforced with the use of statins.[182] Therefore, the effects of prescribing statins to SARS-CoV-2 patients need to be analyzed in a longer term studies.

4.2 | AST, ALT, and GGT

4.2.1 | General aspects

Both AST and ALT are cytosolic enzymes, but the former can also be found in mitochondria in the liver, and GGT is a membrane enzyme.[183] Therefore, elevated AST and ALT levels are used as biomarkers of abnormal liver injury that can be related to chronic viral hepatitis,[184] and GGT indicates liver diseases as well.[185] Usually, high levels of AST, ALT, and GGT are related to mortality.[185]

4.2.2 | In SARS-CoV-2 infection

High levels of the liver enzymes GGT, ALT, and AST (Figure 4) and liver injury can be associated with COVID-19, mainly during hospitalization, and can be used as a biomarker of the disease progression to severe pneumonia, multiorgan failure and death. Besides, the association frequency between those liver enzymes and COVID-19-liver injured patients varied, being high levels of GGT and ALT more frequent than those of AST.[186] However, proteomic analysis has revealed that only AST would be significantly increased in severe COVID-19 patients, when compared to nonsevere patients.[35]

Damaged hepatocytes have also higher odds of progressing to severe COVID-19. Furthermore, severity in COVID-19 cases can be associated with the administration of nonsteroidal anti-inflammatory drugs, herbals and IFN during hospitalization, and thus, these practices should be well monitored. Indeed, after adjustment for age, sex, epidemiological history, liver comorbidities, and initial symptoms, abnormality or liver injury, the use of these drugs was no longer significantly associated with a higher risk of liver injury, except for lopinavir and ritonavir.[186]

It is also important to point out that liver damage can be caused by bile duct obstruction as well and in the context of SARS-CoV-2 infection, this region could be a target too, for its lining cells express ACE2, which could reinforce the need for monitoring liver enzymes and SARS-CoV-2 levels in liver injured patients.

4.3 | Lactate dehydrogenase

4.3.1 | General aspects

The LDH is an intracellular enzyme produced in the liver, pancreas, kidneys and heart, among other organs, and is responsible for converting pyruvate into lactate, by oxidation of NADH to NAD^+. Some diseases are known for lesioning the cells and releasing the enzyme in the plasma and, consequently, decreasing the oxygenation level once the glycolytic pathway becomes upregulated. Therefore, if lactate levels rise, the acidification of extracellular pH leads to the activation of macrophages, for instance. This scenario can occur, for example, in lung cells, which also present LDH-isoenzyme.[172]

4.3.2 | In SARS-CoV-2 infection

High LDH levels (Figure 4) are associated with worse outcomes of SARS-CoV-2 infections, which can lead to mortality. It is important to highlight that some differences can appear regarding the LDH levels and COVID-19 outcomes, and multicentric analyses. Protocol standardization is still necessary.[172]

4.4 | Glyceraldehyde 3-phosphate dehydrogenase

4.4.1 | General aspects

The GAPDH is a cytoplasmatic oxidoreductase[187] ubiquitously expressed in the human body, and can be found in homologous tetramer, dimer and monomer, which have respectively 148, 74, and 37 kDa.[188] Besides its role in glycolysis, GAPDH can also be translocated to the nucleus and interact with DNA and RNA. This translocation to the nucleus is due to posttranslational modifications, such as lysine-acetylation and nitric oxide-induced carbonylation, and inhibition of its glycolytic activity by S-nitrosylation of the cysteine residue housed in the

catalytic site. In fact, GAPDH can be related to apoptosis, both in positive and negative ways,[189] and regulation of the telomere length,[187] which is responsible for protecting chromosome ends.[190]

GAPDH can also be used as a biomarker of vascular endothelium function[191]; inhibition of methionine aminopeptidase-2 (MetAP2)[192]; and of neurodegenerative Alzheimer's Disease progression.[193] In addition, during viral infections, GAPDH can bind to viral RNA sequences that are not translated and eventually inhibit their transcription.[194]

4.4.2 | In SARS-CoV-2 infection

It has been hypothesized that SARS-CoV-2 infection follows the model of infections by SARS-CoV and MERS-CoV.[24] It is suggested that the inhibitory activity caused by MERS-CoV is extended to both newly synthesized mRNA and the previous one in the cytoplasm. Indeed, MERS-CoV and SARS-CoV engendered the reduction of endogenous mRNAs levels of GAPDH.[195] However, proteomic analysis indicated that SARS-CoV-2 infection can increase the expression of enzymes related to the glycolytic pathway, such as GAPDH (Figure 4) and hexokinase.[171] The inhibition of hexokinase with 2-deoxy-_D_-glucose has led to the prevention of the viral replication in Caco-2 cells,[171] and therefore, GAPDH inhibition may prevent the infection as well.

5 | IMPACT OF COVID-19 ON ENZYME MARKET

As expected, the worldwide COVID-19 pandemic directly affected the global market. However, analyzing specifically the enzyme market, the projections still indicate a tendency of growth even in this critical moment.[22] Indeed, Global Market Insights[196] projections, reported in January 2019 that the enzymes market size was around USD 6.3 billion in 2017, exhibiting an estimated growth rate of 6.8% CAGR (compound annual growth rate) up to 2024. According to the Grand View Research report (2020),[197] the enzyme market was estimated at USD 9.9 billion in 2019, with a CAGR of 7.1% until 2027. Thus, optimism for the enzyme market is observed, since not only is expected to stabilize, but it exhibits a growth rate for the upcoming years, being expected to reach USD 13.95 billion in 2024. Even a more conservative report, published by Markets and Markets in April 2020,[198] highlights the potential increase of the enzyme market, expected to reach USD 5.9 billion by the end of 2020 and USD 8.7 billion in 2026.

This optimistic scenario can be associated with the increasing use of enzymes in protein engineering technology and high opportunities in untapped developing countries. The great advancements in biomolecular technologies, a greater concern regarding the development of more sustainable processes, and the increasing use of such proteins in foods, especially in the search for functional and bioactive ingredients; biofuels; pulp and paper industry; and personal care and cosmetics, have boosted this area. The identification, purification and characterization of relevant enzymes and metabolites and also the advent of recombinant DNA techniques enable the achievement of high reaction yields, on a large scale and at relatively low costs.[198–200] In addition, the improvements regarding the enzymatic characteristics, such as stability and specificity[200] have expanded the possibilities of applications of such biocatalysts in various sectors.[201]

In this pandemic moment, the observed growth is also associated with the use of enzymes in diagnosis, in pharmaceutical engineering[197] for the obtainment of new drugs, as presented in the previous sections, and detergents. There is also a trend in the pharmaceutical industry to produce enzyme-based drug formulations, once they generally present high purity degrees, exhibit low side effects and can be delivered to targeted cells.[197] This growth is evident when the numbers of patents are analyzed. As expected, most patents (78.49%) were related to "Drugs," to the obtention of different products (vaccines, new viral proteins, mixtures, or combinations of active ingredients, among others). It was possible to identify that 21.35% encompass "Enzymatic Detergents" and only
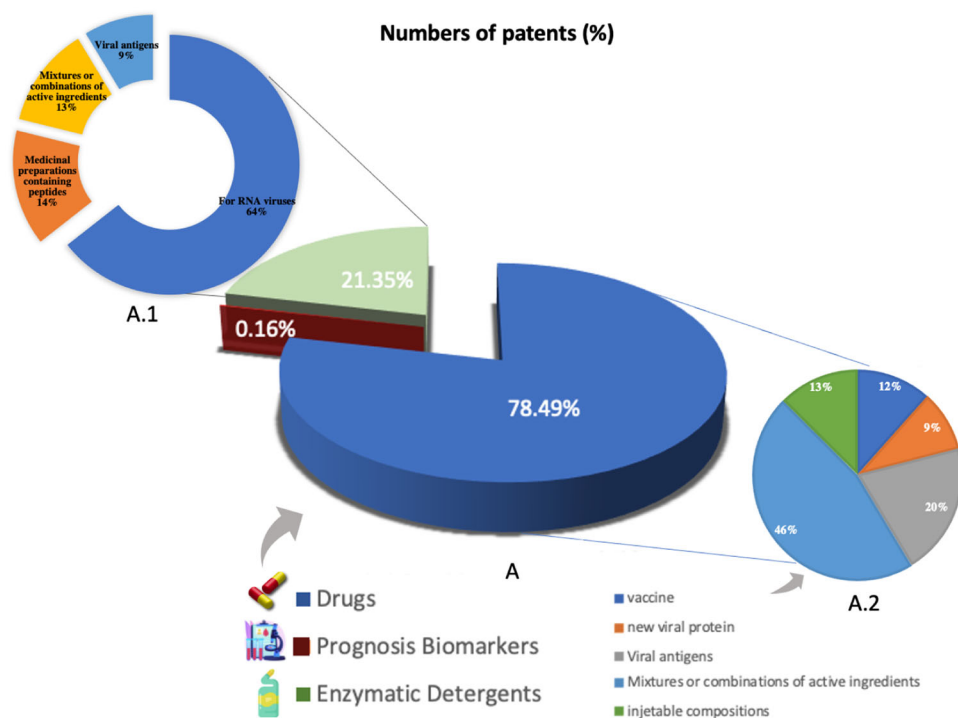
FIGURE 5 Profile of the patents found in "patentinspiration" platform: (A) Overview of the numbers of patents (%) found for "Drugs," "Prognosis Biomarkers," and "Enzymatic Detergents," employing the keywords: "COVID-19" and "enzyme" and "drugs"; "COVID-19" and "enzyme" and "prognosis biomarkers"; "COVID-19" and "enzyme" and "enzymatic detergents"; (A.1) application profile within enzymatic detergents category; (A.2) application profile within drugs category. COVID-19, coronavirus disease 2019 [Color figure can be viewed at wileyonlinelibrary.com]

0.16% "Prognosis Biomarkers" within the same universe (COVID-19 and enzyme) (Figure 5). The data were compiled using the patenteinspiration. com platform, with the keywords: "COVID-19" and "enzyme" and "drugs"; "COVID-19" and "enzyme" and "prognosis biomarkers"; "COVID-19" and "enzyme" and "enzymatic detergents" (December 15th, 2021).

As presented in the previous sections, many of the drugs used or suggested to treat COVID-19 nowadays are repurposed molecules, but there is still room for the development of new drugs (as summarized in Figure 6). Moreover, it is important to develop molecules that act specifically on SARS-CoV-2 with reduced or none adverse reactions. Then, the enzyme market can benefit from all those opportunities to expand product offerings and grow stronger, while contributing to the overcome of COVID-19.

6 | COVID-19 AND ENZYMES: THE GREAT GAP FOR DEVELOPMENT OF NEW DRUGS

Every day that the SARS-CoV-2 pandemic extends, the need for a sharp treatment protocol becomes more and more obvious, and the gap led by the development of new drugs gets clearer. However, it is not due to a lack of efforts, inasmuch as worldwide scientists have been joining resources and sharing intelligence to overcome COVID-19. It was shown that both viral and host enzymes are crucial molecular targets in the course of SARS-CoV-2 infection.[7,24] Host proteases,[5–7,12,25] kinases[29,30] and other yet-poorly studied enzymes[31–36,38] are subverted by
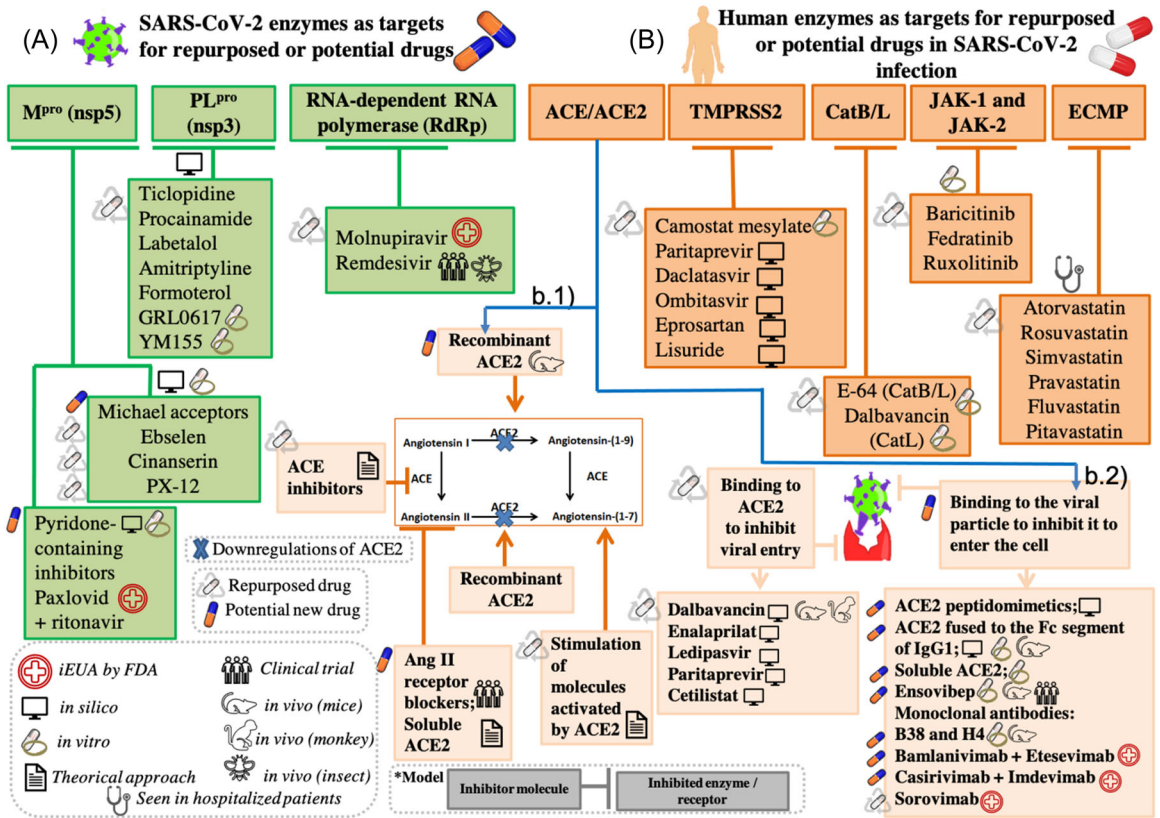
FIGURE 6 Landscape of the potential drugs that target human enzymes and SARS-CoV-2 enzymes, besides new drugs to treat COVID-19. Most of the drugs proposed to treat SARS-CoV-2 are repurposed drugs that target viral enzymes (A) or human enzymes (B), although potential new drugs also rise. Regarding the development steps, some of the molecules are yet a theoretical approach and most of them are still in scan phase (in silico approaches) or in preclinical phase (in vitro or in vivo analysis), while a few molecules are already in clinical trials with COVID-19 patients. All those steps, from the in silico studies to the clinical trials, are crucial before prescribing a drug, even a repurposed one, or a potential new drug to patients. Restoring ACE/ACE2 balance is one of the pharmacological approaches to treat COVID-19, due to the systemic symptoms worsening caused by downregulation of ACE2 in lungs and monocytes, and to upregulation of the enzyme in pancreas. Considering the lower levels of ACE2, there are five ways that one can restore ACE/ACE2 balance, (b.1) providing a molecule that acts on the reaction axes coregulated by ACE and ACE2 (ACE inhibitors; reducing Ang II activity by providing Ang II receptor blockers or soluble ACE2; stimulating molecules activated by ACE2, through Ang-(1–7) agonists or providing exogenous ACE2 to the body, such as recombinant ACE2); and (b.2) administering a molecule that prevents SARS-CoV-2 from attaching to ACE2, by binding to the viral particle or to ACE2. It is worth to remember, however, that prescribing drugs to restore ACE/ACE2 balance should consider ACE/ACE2 balance in pancreas as well. In this context, since in pancreas, ACE2 in pancreas is upregulated instead of downregulated as in the lungs and monocytes, a drug prescribed to restore ACE/ACE2 balance may lead to opposite results in each of those organs or cell, and then, continuous monitoring of the organ functions is needed. Symbols: The symbols above each box correspond to the general study of each group of molecules. The symbols on the left of each molecule or box comprise the classification in new drug or repurposed drug. If a specific molecule of a given group was submitted to a distinct study type, differing from the other molecules of the group, other symbol is added to the right of the respective molecule. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme-2; Ang II, angiotensin II; Ang-(1–7), angiotensin-(1-7); CatB/L, cathepsins B and L; CatL, cathepsin L; ECMP, enzymes from the cholesterol metabolism pathway; EUA, emergency use authorization; FDA, Food and Drug Administration. iEUA, issued as emergency use authorization by the FDA; IgG1, immunoglobulin G-1; Jak, Janus kinase and which is part of the Janus kinase (JAK)/signal transducer and activator of transcription proteins (STAT) signaling pathway; M^pro, main protease; nsp, nonstructural protein; PL^pro, papain-like protease; RdRp, RNA-dependent RNA polymerase; TMPRSS2, transmembrane serine protease-2 [Color figure can be viewed at wileyonlinelibrary.com]

the virus, and host lipase and amylase,[54,177] transferases[6,35,186] and dehydrogenases[171,172] levels, as well as enzymes from the cholesterol metabolism pathway,[171] may reflect the type of response to the infection.

In addition, the development of effective drugs to treat COVID-19 patients can benefit directly from structural and activity knowledge about the molecular targets.[7,26–28,40,44,94,98–100,151,153,166,169] Information on how is the host-virus interaction in terms of protein binding[5,7,25,31,32,49,50] is also valuable. Since the drug targets in SARS-CoV-2 infection belong to the host[5–7,12,25,31–36,49,50] or are a viral enzyme that has structural similarities with a host homologue,[169] one shall be careful in not deregulating even more the cell functioning, or at least aim to minimize the treatment risks.

The importance of enzymes to the viral replication cycle, such as proteases,[26,27] but not only them,[29–36] and the favorable projections on the enzyme market during this pandemic[22,196–198] suggest that choosing enzymes as drug targets to overcome SARS-CoV-2 with a structural approach has the potential to lead us to win this battle. Hence, the pandemic opens new perspectives for the development of new drugs and for the repurposing of others. The use of repurposed drugs aims to facilitate the identification of already commercialized molecules to treat a disease that can be redirected to treat another malady,[168] considering that drug discovery is a slow process.[202] The identification of the molecule is generally done through computational screening,[203] that is, in silico studies. As a matter of fact, in silico analysis can also be used for the discovery of novel molecules that, for example, can act as enzyme inhibitors.[204] Indeed, in silico approaches can help scientists to find compounds that can fight the infection by SARS-CoV-2.[168] An example worth of mention is the online platform created by Xu et al.[202] Such platform, named Shennong, provides databases about FDA approved drugs, drugs on phase 3 clinical trial, and natural products, and a user-friendly interface that displays the molecules docked on their host or viral targets in the scenario of SARS-CoV-2 infection, among other advantages.[202] Therefore, in the present paper, we discussed both repurposed and potential new drugs at different points of the drug discovery chain, ranging from in silico studies to clinical trials and EUAs issued by the FDA.

Besides, it is important to highlight the development of Paxlovid, an M^pro inhibitor by Pfizer Inc., as previously mentioned,[158] and of molnupiravir (MK-4482/EIDD-2801, also known as Lagevrio) by Merck & Co., Inc and Ridgeback Biotherapeutics.[205]

Molnupiravir is an oral-administered ribonucleoside analog that inhibits the replication step of SARS-CoV-2,[205] as it increases the number of mutations in the viral genomic RNA.[206] As a prodrug,[207] molnupiravir-caused mutagenesis in SARS-CoV-2[208] occurs during the elongation process[209] in two steps: first the active form of molnupiravir (β-D-N4-hydroxycytidine triphosphate) is incorporated in the viral genomic RNA by RNA-dependent RNA polymerase (RdRp), and second the resulting molnupiravir-containing RNA is used as a template for the next replication steps.[208] This leads to the error catastrophe process, which is the propagation and accumulation of errors in the viral genome and culminates in the inhibition of viral replication.[207] Molnupiravir, however, is not a specific prodrug to treat SARS-CoV-2, for it can also be used to treat infections caused by other viruses.[208] In phase 3 clinical trial, molnupiravir reduced the risk of hospitalization or death for patients with mild or moderate COVID-19 by approximately 50% when compared to patients that took the placebo (7.3% of patients who received molnupiravir were either hospitalized or died through day 29 of the analysis versus 14.1% of patients treated with placebo).[205] Recently, EMA has reviewed molnupiravir data and decided that it can be administered in SARS-CoV-2 infected patients who do not need oxygen supply and who are at risk of developing severe COVID-19 symptoms.[210] Moreover, the FDA issued an EUA for molnupiravir on December 23rd, 2021.[211] According to the FDA, molnupiravir is recommended for COVID-19-patients that are at least 18 years old who have mild to moderate infection at risk for progression to severe infection.[211] However, the administration of molnupiravir is not indicated for already hospitalized COVID-19-patients and may cause fetal harm.[211]

Previously, remdesivir, an intravenous-administered RdRp inhibitor[212] also known as Veklury[213] and produced by Gilead Sciences,[209] was pointed out to treat SARS-CoV-2 infection.[212] The FDA approved on October 22nd, 2020 the use of remdesivir to treat adults and older than 12 years old and weighing at least 40 kg pediatric patients infected with SARS-CoV-2 requiring hospitalization,[213] and it could also be coadministered with baricitinib to treat

SARS-CoV-2 infected patients,[30] as previously discussed in this paper. The active triphosphate form of remdesivir is incorporated into RNA by SARS-CoV-2 RdRp, terminating RNA synthesis.[212] Nonetheless, the efficacy of remdesivir is controversial since not only is the drug not associated with survival improvement, but also related to longer hospitalization periods.[214] Hence, on February 4th, 2021, the FDA communicated its concern about the potential risks for patient exposure to substandard compounded remdesivir.[213] This concern is because remdesivir is difficult to be produced due to its pH stability and limited aqueous solubility.[213] Besides, remdesivir has six stereocenters and, therefore, it has polymorphic forms.[213] These characteristics could contribute to substandard dose and/or product quality.[213] Then, the FDA alerted compounders to be cautious with the supplies used to produce remdesivir and to use only FDA-approved drugs.[213]

Regarding the possibility of nonsurvival improvement and extended hospitalization period related to the administration of remdesivir, it may be due to its six stereocenters. It would be of interest that remdesivir isomers were analyzed through quantitative structure-activity relationship, to find out which isomer(s) is(are) important for the biological activity of the drug and then synthesis of this(these) isomer(s) could be studied separately. This should be possible through enzymatic catalysis, once biocatalysts are known for their regio-,[215,216] enantio-,[217–220] and chemoselectivity,[221] and therefore purity would be improved contributing to standard dose administration.

Although SARS-CoV-2 RdRp was not discussed in the present work, it is important to mention molnupiravir as well as the M^pro inhibitor Paxlovid because, as mentioned above, the drugs were issued by the FDA as EUA,[159,211] and Paxlovid is under analysis by EMA,[161] whereas molnupiravir was already approved by the organ.[210] Also, the case of remdesivir[213,214] highlights the need for continuous development of new drugs against the virus. The production of oral drugs is encouraged because they can be easily administered in nonhospitalized patients and are less expensive than the antibodies already authorized to be used by nonhospitalized individuals.[209] These aspects reinforce the importance of structural enzymatic studies in the context of COVID-19 as a way to rationalize the development of molecules that can target SARS-CoV-2,[208] as both direct enzymatic inhibitors and as substrates that can cause enzyme malfunction. It is important to emphasize as well the need for deeper studies on less discussed enzymes that may be involved in SARS-CoV-2 infection, to provide a broader view about the viral mechanism of action, which may lead to possible new drugs targets.

In addition, choosing a drug target to treat SARS-CoV-2 infection should concern potential mutations to evade the drug.[209] The S protein, for example, can house different mutations, as seen for the Omicron variant, whereas M^pro and RdRp targeted by Paxlovid and molnupiravir respectively are less likely to mutate, even for Omicron variant.[209] Thus, it is crucial for the development of new drugs that not only structural studies on SARS-CoV-2 enzymes are conducted, but also continuous monitoring on their nucleotide sequence and possible mutations should take place. Truly, it is critical that alterations in SARS-CoV-2 nucleotide, protein and metabolite profiles be confronted so that the most significant alterations in the viral mechanism of action can be established. Then, it can be analyzed if those alterations interfere in the drug's mechanism of action, or even if they interact with host enzymes differently from what was previously known.

In this scenario, the importance of multiomics sciences is highlighted. COVID-19 is a very complex disease that can have devastating results. After 2 years since the SARS-CoV-2 pandemic started, worldwide scientists are just beginning to understand the extension of the infection.

On the one hand, great amounts of datasets have been acquired, such as genomics,[6,8,130,222] transcriptomics,[33] proteomics,[31,35,36,171] metabolomics,[35] interactomics.[36] Glycomics[223] and lipidomics[224–226] were also exploited. Enzyme structure and possible inhibitors were also investigated.[5,16,26,27,156]

On the other hand, each of those studies scrutinizes just one type of dataset, which means one aspect of SARS-CoV-2 biology or one way of interaction with the host, and a broader view is necessary. Indeed, cell events are not isolated and a myriad of molecules are constantly being produced and recycled in the cell. Besides, these dynamics can change during infection. Concerning enzymes specifically, both viral and human ones, it is important that they be investigated in omics studies to their complex roles in COVID-19. In fact, as previously discussed in the present

paper, there are many enzymes whose roles in SARS-CoV-2 infection are yet to be discovered or more deeply analyzed.

Multi-omics approaches regard different knowledge layers,[227] providing a scenario of cell events during infection and of the viral biology itself, instead of a limited perspective obtained when just one type of analysis is done. Multi-omics methods can be used to provide information on the viral mechanism of action, on the interaction with host cells, and on the correlation among virus genotyping, host enzymes and disease severity/prognosis. This can contribute to the understanding of COVID-19 and to the development of effective drugs against SARS-CoV-2 in the future.

In the COVID-19 context, enzymes are essential. The discoveries of SARS-CoV-2 enzyme structures and of how they influence host enzymes are some of the driving forces of scientists worldwide to repurpose drugs and then to develop specific inhibitors against the virus. Also, the viral influence in host enzyme levels needs to be monitored, effort for which biochemical tools need to be produced. The commercialization of enzymatic detergents is essential to prevent the spread of the virus to the point that they can degrade the viral particle. These roles are expected to boost growth of the enzyme market and reinforce the prominence of the biotechnological field in the upcoming years, even after we have beaten SARS-CoV-2 and COVID-19.

The overcome of the worldwide SARS-CoV-2 infection will only happen through global massive financial support in science, more specifically in biotechnology, experts from different fields sharing their findings, and commitment of the global community to follow public health protocols based on evidence rather than believing in hasty and distorted information.

7 | FINAL CONSIDERATIONS AND PERSPECTIVES

The great effort of the worldwide scientific community to understand the SARS-CoV-2 mechanism of action and how it subverts the human body machinery; to develop an effective vaccine and rapid low-cost diagnosis devices; and to establish a robust drug therapy is noteworthy. All these aspects reflect in the significant increase in publications about COVID-19 during this pandemic period, which reinforces the importance of the role of science in the world. Indeed, the established protocols against the virus have been structured based on the studies about SARS-CoV-2 molecules and the correlations of the infection course and clinical enzymes identified so far.

Actually, enzymes have been pointed out as flagships on the comprehension of the SARS-CoV-2 mechanism of infection, playing crucial roles in the virus replication cycle and in its binding to host cells, which enables the process of the virus genome and the immune system invasion. Among them, proteases play a central role, but others, such as kinases, transferases, amylase, lipase, and dehydrogenases, are important as well; whereas others deserve to be further studied, such as deaminases and ligases, for they may take part in SARS-CoV-2 infection too. Moreover, enzymes are attractive biomolecules to functionalize specific drugs to target cell delivery. In this context, biotechnology stands out as a promising area.[228] Indeed, Dr Jeremy Levin, president of the Biotechnology Innovation Organization (BIO) brought together more than 40 key opinion leaders to write a book entitled "Biotechnology in the Time of COVID-19,"[229] in an attempt to compile the advances achieved in this field during the pandemic and to state the challenges that have been faced by the biotechnology industry.

From all that has been discussed, it is expected that biotechnology will play an even more important role in upcoming years. Therefore, understanding the role of enzymes is essential: to understand the viral mechanism action, to develop new drugs, and to produce new diagnosis devices. The increasing importance of the enzymes directly reflects on the enzyme market and its tendency to grow, even during a global economic crisis. The SARS-CoV-2 tragic experience, which revealed itself as a major challenge that on October, 2020 had already costed more than the Iraq War to the United States, reaching more than $16 trillion,[230] has highlighted the importance of a united scientific community and the need to establish robust interdisciplinary partnerships.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ORCID

Luana Xavier Soares Gomes Moura Fé http://orcid.org/0000-0001-6402-1810

Eliane Pereira Cipolatti http://orcid.org/0000-0002-3272-712X

Martina Costa Cerqueira Pinto http://orcid.org/0000-0001-7412-3626

Suema Branco http://orcid.org/0000-0003-2871-1915

Fábio César Sousa Nogueira http://orcid.org/0000-0001-5507-7142

Gisela Maria Dellamora Ortiz http://orcid.org/0000-0002-5640-2194

Anderson de Sá Pinheiro http://orcid.org/0000-0001-5118-6931

Evelin Andrade Manoel http://orcid.org/0000-0002-9295-2644

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AUTHOR BIOGRAPHIES

Luana Xavier Soares Gomes Moura Fé is graduated in Pharmacy from the Federal University of Rio de Janeiro (UFRJ, Brazil) (2017) and has Master's degree in Pharmacy from Postgraduate Program in Pharmaceutical Sciences (PPGCF) at UFRJ. Currently, she is PhD student (since 2019) in Pharmaceutical Sciences from UFRJ and work in the Pharmaceutical Biotechnology Department at UFRJ. She has experience in Medical Biochemistry and Genetics, and has experience as a monitor of Analytical Chemistry at the Institute of Chemistry at UFRJ and of Pharmaceutical Biotechnology at the Faculty of Pharmacy at UFRJ. Her areas of expertize include Pharmaceutical Biotechnology with emphasis on Biocatalysis and Immobilization of Lipases, Purification and Structural Analysis of Enzymes and Proteins and Proteomics.

Eliane Pereira Cipolatti is a Food Engineer and has a PhD in the area from the Federal University of Santa Catarina (UFSC, Brazil). Part of her thesis was developed at the Consejo Superior de Investigaciones Científicas in Madrid

(Spain). Her thesis was defended in 2015 and received an honorable mention awarded by the Coordination for the Improvement of Higher Education Personnel, a foundation of the Ministry of Education, being considered one of the best theses in the country in the area of Food Science. She worked as professor at the UFRJ (2019–2021) and currently is a permanent professor at the Federal Rural University of Rio de Janeiro (UFRRJ). She participates of the editorial board of Frontiers in Catalysis in the area of Biocatalysis. She has experience in the area of Food Science and Technology, of Enzyme Technology and Bioprocesses, and she works mainly in the production of enzymes by solid and submerged culture, in immobilization of enzymes, in production of bioactive esters and in the study of antioxidant activity of carotenoids and phenolics compounds (index H = 15).

Martina Costa Cerqueira Pinto has a PhD in Chemical Engineering from the Engineering Chemistry Program (The Alberto Luiz Coimbra Institute for Graduate Studies and Research in Engineering-COPPE) at UFRJ, (2017) and a Master's degree in Chemical Engineering from the Chemical Engineering Program at COPPE/UFRJ (2013). She carried out research activities in the area of functionalization of polymeric materials during the Sandwich Doctorate at Queen Mary University of London in 2016. Currently she works as a Post-Doc at COPPE/UFRJ uniting the Polymerization Engineering and Biotechnology areas. She won the first place in the Shell Youth Initiative 2018 Program, Shell and CIEDS (index H = 9).

Suema Branco is currently a collaborating researcher in research projects at the Laboratory of Toxicology at the Biophysics Institute at UFRJ, and at the Phytology Laboratory of the Botany Department of the National Museum/UFRJ. She was a professor in the undergraduate course in Biological Sciences at the Federal University of Rio de Janeiro. She has a doctorate and a Master's degrees in Biological Sciences from the National Museum/Federal University of Rio de Janeiro. She took the Bachelor's Degree in Biological Sciences at the Federal University of Rio de Janeiro. She has experience in microorganism Cultivation, Environmental Monitoring of Marine, Continental philogeny (index H = 5).

Fábio César Sousa Nogueira holds a bachelor's degree and a degree in Biological Sciences from the State University of Ceará (UECE). He holds a Master's degree in biochemistry from the Federal University of Ceará (UFC), Department of Biochemistry and Molecular Biology, and a doctorate in Biochemistry from the Federal University of Rio de Janeiro (UFRJ), Institute of Chemistry (IQ), Department of Biochemistry, with a sandwich doctorate at University of Southern Denmark, Department of Biochemistry and Molecular Biology, Protein Research Group. He did a postdoctorate at the Biochemistry Department, IQ, UFRJ, where he also worked as a visiting professor. He has a h = 18 factor. He is a founding member of the Brazilian Proteomics Society (BrProt), where he worked as treasurer from 2012 to 2014. He's currently an adjunct professor at the Department of Biochemistry, IQ, UFRJ and a member of the Proteomics Unit (IQ/UFRJ), of the Brazilian Doping Control Laboratory (LBCD) and of the Technological Development Support Laboratory (LADETEC). His expertise area is Biochemistry, with an emphasis on Proteomics and on Plant Proteomics, and he works mainly on: mass spectrometry, liquid chromatography, uni and bidimensional electrophoresis of proteins, post-translational modifications (glycosylation, phosphorylation, and acetylation) and quantitative proteomics (without label and dependent on the label). He also has experience in the analysis of peptides and proteins used in doping by liquid chromatography (LC) and nanoLC coupled to the mass spectrometer. He worked in the preparation of the LBCD for the Rio2016 Olympic and Paralympic Games and participated in the analysis during the games (index H = 21).

Gisela Maria Dellamora Ortiz is graduated in Pharmacy from the Faculty of Pharmacy of the Federal University of Rio de Janeiro (1978), master's degree in Biochemistry from the Institute of Chemistry at the Federal University of Rio de Janeiro (1983) and doctorate in Biochemistry from the Institute of Chemistry at the Federal University of Rio de January (1991). She did a post-doctorate at the Department of Biocatalysis at the Instituto de Catálisis y Petroleoquímica-CSIC, Madrid, Spain (2004), under the supervision of Professor Jose Manuel Guisan. She is Associate Professor IV at the Faculty of Pharmacy at UFRJ and holds the position of Director of

the Faculty of Pharmacy at UFRJ (2014–2018 term). She is a permanent professor in the Graduate Program in Pharmaceutical Science and Technology (PPGCTF) at the Faculty of Pharmacy at UFRJ. She is a member of the Brazilian Association of Pharmaceutical Sciences (ABCF) and of the Brazilian Society for the Progress of Science (SBPC). She works in area of pharmacy, with an emphasis on industrial enzyme and pharmaceutical biotechnology, coordinating projects, guiding undergraduate, master's and doctoral students, mainly in the following subjects: production and purification of microorganism enzymes, enzyme immobilization, use of free and immobilized lipases, biocatalysis (index H = 15).

Anderson de Sá Pinheiro is graduated in Pharmacy from UFRJ (2000), and has a master's (2003) and a doctorate (2007) in Biological Chemistry from UFRJ and has a postdoctorate from Brown University, USA (2007–2011). He is currently an Associate Professor I of the Department of Biochemistry at the Institute of Chemistry at UFRJ, where he coordinates the Molecular Biochemistry Laboratory (LaBMol). He has experience in structural determination and dynamic characterization of proteins through Nuclear Magnetic Resonance spectroscopy. His research aims to understand the molecular bases of the mechanisms of regulation of gene expression, in particular: (i) RNA-binding proteins and histone methyl transferases as therapeutic targets for cancer; (ii) quorum sensing regulators in bacteria with a view to the development of new antimicrobials. Currently, he worked as coordinator (2019–2021) of the Graduate Program in Biochemistry at the Institute of Chemistry at UFRJ—PPGBq (CAPES 6), of which he was vice-coordinator (2017–2019) and is a permanent member. He has coordinated research projects funded by national (FAPERJ and CNPq) and international (Brown University) agencies. He was a Young Scientist from Our State (FAPERJ) scholarship holder during the 2014–2017 period (index H = 15).

Evelin Andrade Manoel is a Biologist and holds a PhD in the area of Biochemical Processes from the Federal University of Rio de Janeiro (UFRJ, Brazil). Her thesis was presented in 2014 and she was awarded by the Coordination for the Improvement of Higher Education Personnel (c, a foundation of the Ministry of Education, being considered the best thesis in the country in the area of Engineering. She also received the Who's Who in the World in 2014. She works as a permanent professor at the Faculty of Pharmacy at UFRJ. The researcher also collaborates as professor in the Graduate Program in Pharmaceutical Sciences (PPGCF) and in the Master's Course at the Faculty of Pharmacy (CTECFAR), besides in the Graduate Program in Biochemistry at the Institute of Chemistry at UFRJ (PPGBq). She has experience in the areas of Biochemical and Biochemical Engineering with an emphasis on Biotechnology applied to Pharmaceuticals and Cosmetics, acting on the following topics: drugs development, enzymes in therapy, Immobilization of different biocatalysts, Production of bioactive substances, Biocatalysis, Enzymatic kinetics, Reaction optimization in batch reactors and Reactors in continuous flow, Resolution of enantiomers, Ionic liquids and Production of lipases for industrial application. Recently she had a patent filing for the production of a new drug (Process number: BR1020210224649) (index H = 15).

SUPPORTING INFORMATION

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