Targeting the GRP78-Dependent SARS-CoV-2 Cell Entry by Peptides and Small Molecules

Loubna Allam1*, Fatima Ghrifi1*, Hakmi Mohammed1, Naima El Hafidi1, Rachid El Jaoudi1, Jaouad El Harti2, Badreddine Lmimouni3, Lahcen Belyamani4 and Azeddine Ibrahimi5

1Medical Biotechnology Laboratory (MedBiotech), Bioinova Research Center, Rabat Medical & Pharmacy School, Mohammed V University in Rabat, Rabat, Morocco. 2Therapeutic Chemistry Laboratory, Medical Biotechnology Laboratory (MedBiotech), Rabat Medical & Pharmacy School, Mohammed V University in Rabat, Rabat, Morocco. 3Parasitology and Mycology Department, Military Hospital Mohammed V, Rabat Medical & Pharmacy School, Mohammed Vth University in Rabat, Rabat, Morocco. 4Emergency Department, Military Hospital Mohammed V, Rabat Medical & Pharmacy School, Mohammed Vth University in Rabat, Rabat, Morocco.

ABSTRACT: The global burden of infections and the rapid spread of viral diseases show the need for new approaches in the prevention and development of effective therapies. To this end, we aimed to explore novel inhibitor compounds that can stop replication or decrease the viral load of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), for which there is currently no approved treatment. Besides using the angiotensin-converting enzyme (ACE2) receptor as a main gate, the CoV-2 can bind to the glucose-regulating protein 78 (GRP78) receptor to get into the cells to start an infection. Here, we report potential inhibitors comprising small molecules and peptides that could interfere with the interaction of SARS-CoV-2 and its target cells by blocking the recognition of the GRP78 cellular receptor by the viral Spike protein. These inhibitors were discovered through an approach of in silico screening of available databases of bioactive peptides and polyphenolic compounds and the analysis of their docking modes. This process led to the selection of 9 compounds with optimal binding affinities to the target sites. The peptides (satpdb18674, satpdb18446, satpdb12488, satpdb14438, and satpdb28899) act on regions III and IV of the viral Spike protein and on its binding sites in GRP78. However, 4 polyphenols such as epigallocatechin gallate (EGCG), homoeriodictyol, isorhamnetin, and curcumin interact, in addition to the Spike protein and its binding sites in GRP78, with the ATPase domain of GRP78. Our work demonstrates that there are at least 2 approaches to block the spread of SARS-CoV-2 by preventing its fusion with the host cells via GRP78.

KEYWORDS: SARS-CoV-2, Spike, inhibitors, small molecule, peptide, GRP78

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new virus strain of the Coronaviridae family. Coronaviruses (CoVs) are enveloped viruses with a single-stranded positive-sense RNA containing approximately 30,000 base pairs (bps).1 CoVs are involved in many diseases affecting the upper respiratory tract, gastrointestinal system, and central nervous system in humans and animals.1 Two groups of proteins characterize CoVs: structural proteins, including Spike (S), envelope (E), membrane (M) and nucleocapsid (N),2 and nonstructural proteins, such as proteases (nsp3 and nsps5) and RdRp (nsps12).3,4 The CoV S glycoprotein is a precursor protein composed of 1300 amino acids, located on the outer envelope of the virion. It plays an essential role in the attachment, fusion, and entry of the virus into the host cells. Particularly, S protein mediates the fusion of the viral and the cellular membranes5 by binding to different surface receptors of the host cells via its receptor-binding domain (RBD). The transmembrane (S) spike trimeric glycoprotein of the virus consists of 2 functional subunits: S1 and S2. The first, which made up of 4 domains (S1 A, S1 B, S1 C, and S1 D), contributes to the attachment of the virus to the host cell receptor. Then, S2 coordinates the fusion of the 2 membranes.8 This fusion activates proteases and allows the proteolytic cleavage of the S protein.9 The latter causes conformational changes to prime the S2 subunit for the fusion of viral and cell membranes.8 Thus, RBD is the key component by which the virus is translocated inside the cells through its interactions with the angiotensin-converting enzyme (ACE2),10,11 dipeptidyl peptidase-4 (DPP4) and or glucose-regulating protein 78 (GRP78).12

The SARS-CoV-2 RBD contains 13 disulfide bonds that correspond to 13 cyclic regions considered to be similar to the cyclic form of Pep42.13 These regions can interact with the GRP78 cell surface.
The GRP78 or binding immunoglobulin protein (BiP) is a member of the heat shock protein family. It is traditionally regarded as a major chaperone of the endoplasmic reticulum (ER) that facilitate protein folding and assembly, protein quality control, Ca²⁺ binding, and regulating ER stress signaling. When GRP78 moves to the surface of the cell, it can interact with many ligands or other proteins like a multifunctional receptor. It is therefore involved in inflammatory and autoimmune diseases and overexpressed in various human cancers, including prostate cancer, breast cancer, lung cancer, melanoma, and ovarian cancer. In addition to its role in the proliferation, invasion, and metastasis of many cancer cells, GRP78 also has a sensitivity to the recognition of viruses through its substrate-binding domain (SBD) and is involved in the assembly of their envelope proteins. Recently, GRP78 has been recognized as an attachment factor for the Middle East respiratory syndrome coronavirus (MERS-CoV), which improves the entry of the virus in the presence of DPP4, and as one of the SARS-CoV entry receptors in the human cell.

Most research carried out, or measures taken, are reposed on the idea that ACE2 is the primary receptor of SARS-CoV-2. Providing GRP78 as a second receptor in the presence of special physiological conditions when the expression of GRP78 is high, in vivo, it could potentially be more favorable to infection with SARS-CoV-2. Thus, the presence of ACE2 and GRP78 at a high quantity could classify these additional categories to very high-risk types. As there are no efficacy tools to fight against this disease, it is therefore essential to find out how to protect persons with chronic illnesses (cancer, diabetes, and high blood pressure). These people are at greater risk of developing an aggressive form of SARS-CoV-2 infection.

Like SARS-CoV and MERS-CoV, SARS-CoV-2 is a member of Betacoronavirus, which also causes a severe respiratory tract infection with a higher mortality rate. Sequence and structure analysis of S proteins from different CoV strains showed that the specificity of interaction between S proteins and their receptors is the main determinant of the host tropism of these viruses. The main cellular receptors for viral S proteins include ACE2 and DPP4, as well as other molecules that may be involved in the interaction between the virus and the host cell. It has been suggested that GRP78 may also act as another receptor that assists SARS-CoV-2 to penetrate the host cells.

This same proposition has confirmed by Aguiar et al., who concluded that the GRP78-binding site overlaps with the ACE2-binding site, although the residues involved in the interactions may be a little different. For this purpose, a targeted analysis of the expression of candidate genes involved in SARS-CoV-2 infection confirmed the presence of the GRP78 protein in vitro in epithelial cells of the human respiratory tract and lung tissue. This analysis suggests that for ACE2 to be an integral receptor for SARS-CoV-2, there are probably other mechanisms dynamically regulating the expression of ACE2 in the respiratory mucosa in the context of infection with SARS-CoV-2 and/or possibly other co-receptors. These can contribute to the functioning of ACE2 and TMPRSS2 in the binding and fusion of SARS-CoV-2 in this cell type. In the same context, Palmeira et al. have verified that the level of expression of the GRP78 gene is high in the blood of patients with SARS-CoV-2 pneumonia.

The entry of the virus is a key step that can be targeted by a possible therapeutic strategy opting for the prediction of a molecule capable of causing simultaneous inhibition of the 2 proteins. Based on the knowledge gained about the SARS-CoV-2 virus, 2 axes of investigation were explored in this study to prevent the translocation of the virus inside host cells. The first was the inhibition of GRP78 recognition by SARS-CoV-2, by targeting both the nucleotide-binding domain (NBD) and SBD of GRP78 with ATP-competitive small molecules and/or peptides. The second consisted of the inhibition of the viral S protein of SARS-CoV-2 at the GRP78-binding site. Finally, we identified 9 compounds that could act as potential blockers of SARS-CoV-2 cell entry through GRP78 for further consideration as possible therapies against this virus.

Materials and Methods

Ligands retrieval

A library of 100 active phytochemicals (polyphenols) was generated from PubChem database (pubchem.ncbi.nlm.nih.gov). Biologically active peptides were obtained from the Structurally Annotated Therapeutic Peptides Database (SATPdb) (https://doi.org/10.1093/nar/gkv1114).

Targeted docking sites on the GRP78

Two binding domains characterize the structure of GRP78. The first is the NBD that houses ATP, whereas the second (SBD) receives the substrate (peptide or protein) in the form of an excluded segment or partially folded protein. The inhibition of the ATP-binding site interrupts the functional cycle of the protein by modifying its conformation, which may lead to the inhibition of viral penetration.

ATP docking was conducted on GRP78 (PDB ID:5F1X) structure to identify the key residues of its ATPase site. The residues (ASP-34, THR-38, TYR-39, ILE-61, GLU-201, ASP-224, PHE-258, GLY-228, GLY-249, ASP-250, GLY-364, SER-365, ILE-368, and ASP-391) were used as references for the search space determination. Similarly, it was considered advisable to prevent the Spike from being attached to the GRP78 by directly targeting their interaction site. The GRP78 residues involved in Spike binding are Ile-426, Thr-428, Val-429, Val-432, Thr-434, Phe-451, Ser-452, Val-457, and Ile-459 as was previously described.

Target sites on SARS-CoV-2 Spike

According to the study by Ibrahim et al., GRP78 binds to the SARS-CoV-2 Spike in 4 regions, in which regions III

Bioinformatics and Biology Insights
(C391-C525) and IV (C480-C488) showed stronger affinities for the Spike. Consequently, it was proposed in our study to target these 2 regions to prevent any possibility of Spike binding to the GRP78 receptor.

Preparation of molecular docking files

Structures of the GRP78 (PDB ID: 5E84) and SARS-CoV-2 Spike (PDB ID: 6LZG) were downloaded from the RCSB PDB database (http://www.rcsb.org/pdb/home.do) in PDB format. The small-molecule SDF files (molecule.sdf) downloaded in the previous step were converted to PDB format using Open Babel. All inhibitors and receptors have been optimized and converted to PDBQT format using the standard protocol of the Autodock Tools.

Molecular docking

Molecular docking of small molecules (ATP and inhibitors) was performed using AutoDock Vina 1.1.2. Docking of ATP on GRP78 was conducted to determine the key residues of its ATPase site. Docking search space was defined on each targeted site of GRP78 and Spike proteins as explained previously. The ClusPro server was used for peptide-protein docking of the selected peptides. The resulting peptide-protein complexes were ranked by cluster size and visual inspection. Small molecules and peptides were selected for each protein site based on their estimated binding energy and the protein residues with which they are to interact.

Postdocking analysis and visualization

Visual analysis of docking poses and image rendering was performed using PyMOL version 2.3 (Schrodinger, LLC).

Results and Discussion

The global burden of infections and the rapid spread of viral diseases point to the need for new preventive approaches and more effective therapeutic strategies. Therefore, particular priority is being given to the emerging SARS-CoV-2, which is considered a pandemic under current circumstances. In this direction, our study focused on the repositioning of approved drugs as well as the investigation of other bioactive compounds that may prevent the penetration of SARS-CoV-2 into host cells by targeting the region of GRP78 that is required for the interaction with the Spike protein of the virus. Two factors have guided our compound selection process. The first is the identification of peptides that can block the interaction of the SARS-CoV-2 Spike and GRP78, and the second is the screening of small molecules suitable for docking inside GRP78’s ATP-binding pocket to inhibit its activity and thus its interaction with the Spike.

The effect of the selected phytochemicals and peptides on SARS-CoV-2 Spike protein and GRP78 was elucidated by molecular docking analysis. The promising candidates were selected based on their binding mode and affinity against the 2 targets.

Table 1. Set of peptides selected to inhibit spike-SARS-CoV-2 and GRP78.

| PEPTIDES ID | SEQUENCE       | FUNCTION               |
|-------------|----------------|------------------------|
| satpdb12488 | PTTFMLKYDENITTDVDC | Antiviral, antimicrobial |
| satpdb14438 | SNNTAIPTNFSISITTEVM | Antiviral, antimicrobial |
| satpdb28899 | RDSVDFTSVDRDKSTMEL | Antiviral, antimicrobial |
| satpdb18674 | QYGSFCTQLNRALSIGIAEQ | Antiviral, antimicrobial |
| satpdb18446 | VLYNSSFSTFKCYSATK  | Antiviral, antimicrobial |

Peptide–protein docking

Peptide–protein docking performed between the peptides and their designated targets. The peptides docked against the Spike protein and its binding sites in GRP78. Results from their binding modes analysis were favorable, indicating that the selected compounds could potentially inhibit or prevent the entry of the virus. The sequences of the selected peptides are presented in Table 1.

The peptide satpdb18674 selected to target the Spike protein was mainly bound to region IV (C480-C488), whereas satpdb12488 and satpdb28899 showed more affinity to region III (C391-C525) (Figure 1). Thus, this could directly interfere with the Spike/GRP78 interaction as both regions are necessary for the Spike to recognize the GRP78.

Peptides reported as inhibitors of GRP78 (satpdb18674, satpdb18446, satpdb12488, satpdb14438, and satpdb28899) were mainly bound to the region 1426-1459 (Figure 2). This region of GRP78 appears to be critical for the Spike to attach to the host cell.

These results revealed that the peptides satpdb18674, satpdb12488, and satpdb28899 had strong affinities simultaneously with the Spike and with both regions of GRP78 (regions III and IV). We therefore hypothesize that these peptides can prevent the entry of the virus by binding either to regions III and IV of the Spike or to their binding site in GRP78.

The peptide satpdb18674 was particularly bound to the key residues (Figure 3) at the GRP78 target site (VAL-429,
GLN-449, and SER-452) and to those in the region IV of the Spike (CYS-480, ASN-481, and PHE-486). This region showed a strong affinity for GRP78, reason why we consider satpdb18674 to be the most appropriate peptide to block any potential binding of the Spike protein to the host cell via GRP78.

Analysis of the molecular interactions of the peptides stabilizes the target protein by interacting with the residues of their different binding pockets. Several hydrogen bonds have been observed, ensuring strong bonds with the spike binding site residues (regions III and IV) and with those of GRP78.
Table 2 illustrates the details of the interactions between the proteins and peptides selected.

Small molecules and protein docking

The screening of polyphenols was performed against the Spike protein, its binding site in GRP78, and the ATPase domain of GRP78. Postdocking analysis showed favorable binding modes, indicating that the compounds epigallocatechin gallate (EGCG), homoeriodictyol, isorhamnetin, and curcumin could bind simultaneously to the 3 sites.

The 4 polyphenols have established multiple hydrogen bonds with the residues of the GRP78 pocket (Figure 4). Also, these compounds showed high affinity toward the regions III and IV of the Spike protein (Figure 5). The docking results of the compounds EGCG, homoeriodictyol, isorhamnetin, and curcumin in the ATP-binding site of GRP78 showed their favorable positioning inside the receptor’s cavity, mimicking ATP in its mode of interaction with the ATPase domain of GRP78 (Figure 6) with binding affinities higher than or close to that of ATP. Therefore, these compounds could potentially interfere with the virus entry to the host cells. Finally, as CoVs acquire the ability to bind to multiple receptors, and cross the interspecies barrier, this work could provide new insights in the development of effective therapies against SARS-CoV-2 infections.

The set of interactions between the small molecules and the active sites of the 2 targets (ATPase domain, GRP78/spike interaction sites) is summarized in Table 3. The best affinity was attributed to EGCG at all the interaction sites. Binding to EGCG regularly folds the protein while ATP binding unfolds the protein. A slightly unfolded form is the native functional form of GRP78. Thus, due to the link of the EGCG and the movements with SBD, GRP78 will not be able to perform its appropriate functions given the changes in conformation. This may be one of the likely mechanisms by which the EGCG inhibitor acts on the full-length GRP78 protein. The several researches show that polyphenols as an antiviral and anti-inflammatory agent that can be helpful for both prevention and treatment of new emerging CoV. However, well-designed clinical trials are needed to demonstrate the potential efficacy of curcumin against SARS-CoV-2 infection and its ensuing complications.

Current drugs have limited efficacy in the treatment of SARS-CoV-2, given the high number of deaths caused by this virus. Designing new drugs that target specific activities of this virus or stop the stages of its infection cycle is a crucial step. Due to the diverse behavior of SARS-CoV-2 depending on the population type, the production of an appropriate vaccine against this disease is difficult, hence the need for new therapeutic strategies, proposing compounds affecting different stages of the virus’s life cycle, and strategies that target the entry of the virus on the ACE2 side and others on the SARS protease side. Our study will complement these strategies by targeting the different sites involved in the SARS/GRP78 interaction (Figure 7). The neglect of the latter causes a high risk of infections. The set of molecules (4 polyphenols and 5 bioactive peptide) was selected in this study to inhibit the SARS-CoV-2 Spike/GRP78 interaction. Satpdb18674 and EGCG gave the best results for all the targeted sites for the 2 proteins. Our results, therefore, meet an urgent need, given the absence of an effective drug available. As demonstrated in this combined approach, screening and reassigning bioactive molecules to prevent the entry of this virus on the Spike/GRP78 side can provide an accelerated approach to identify and develop new treatments for SARS-CoV-2 infection.

Inhibition of the interaction between the spike protein SARS-CoV-2 and the receptor by blocking the GRP78 is a strategy interesting to identify drugs that decrease the rate of viral infection. Several studies have given confirmatory evidence to the presence of the GRP78 protein in patients infected with SARS-CoV-2. In addition, GRP78 is part of receptors that allow the virus to enter and facilitate the initial infection of host cells, the reason why it has become a receptor for SARS-CoV-2 and the GRP78 protein could be a receptor for SARS-CoV-2 and to propose molecules capable of hindering this interaction. Reducing the level of expression or inhibition of GRP78 by small interfering RNAs or by siRNAs, respectively, blocked the entry of Japanese encephalitis virus and Ebola virus.
Table 2. Recapitulating table details the size and bonds types of different residues between the targets (SARS-CoV-2 peak, GRP78) and the peptides (satpdb18674, satpdb18446, and satpdb12488, satpdb14438, and satpdb28899).

| PEPTIDE   | TARGET | PEPTIDE | GRP78 | SIZE (Å) | INTERACTION TYPES | PEPTIDE | SPIKE | SIZE (Å) | INTERACTION TYPES |
|-----------|--------|---------|-------|----------|-------------------|---------|-------|----------|-------------------|
| Satpdb12488 | ASP-19 | THR-434 | 2.1; 1.8; 2.6 | O-NH; OH-O; O-O | MET-5 | TYR-473 | – | 1.8; OH-O |
| THR-2 | VAL-429 | 2.5 | NH-O | PHE-4 | TYR-473 | 3.2 | OH-O |
| ASP-16 | LYS-447 | 1.8; 2.7 | NH-O; N-O | THR-13 | TYR-453 | 2.8 | OH-O |
| ALA-17 | LY-447 | 3.4 | NH-NH | ASP-16 | TYR-453 | 3.8 | OH-O |
| MET-5 | SER-452 | 1.8; 2.6 | OH-O; O-O | THR-13 | ARG-403 | 2.2 | NH-O |
| | | 2.3; 3.9 | NH-O; O-O | THR-2 | ALA-475 | 3.4 | O-O |
| PHE-4 | SER-452 | 2.3 | NH-O | THR-3 | ALA-475 | 3.7 | O-O |
| TYR-15 | | | | GLU-10 | LYS-417 | 3.0 | O-NH |
| Satpdb14438 | ILE-13 | THR-434 | 1.9 | OH-O | – | – | – | – |
| | | | | | | | | |
| SER-12 | VAL-429 | 1.9; 2.9 | NH-O; O-O | | | | |
| THR-9 | THR-458 | 2.2; 3.1; 3.3 | NH-O; N-N-O-O | | | | |
| Satpdb18446 | TYR-3 | THR-434 | 3.4 | O-OH | – | – | – | – |
| ASN-4 | VAL-429 | 1.9; 2.8 | NH-O; O-N | | | | |
| TYR-3 | GLN-449 | 2.6; 1.9; 3.6 | NH-OH; NH-O | | | | |
| VAL-1 | SER-452 | 3.9 | NH-NH | | | | |
| THR-10 | SER-452 | 1.9; 2.8 | O-OH; O-O | | | | |

(Continued)
Table 2. (Continued)

| PEPTIDE | TARGET | INTERACTION RESIDUES | INTERACTION TYPES | PEPTIDE | SPIKE | INTERACTION RESIDUES | INTERACTION TYPES |
|---------|--------|----------------------|------------------|---------|------|----------------------|------------------|
|         |        | PEPTIDES-GRP78       |                  |         |       | PEPTIDES-SPIKE       |                  |
|         |        | INTERACTION SIZE (Å) |                  |         |       | INTERACTION SIZE (Å) |                  |
|         |        | INTERACTION TYPES   |                  |         |       | INTERACTION TYPES   |                  |
|         |        |                      |                  |         |       |                      |                  |
| Satpdb18674 | GLN-1 | VAL-429              | 2.5; 2.5; 2.9    | NH-NH; 2H-N | SER-4  | --                  | PRO-479          | 1.9; 2.8         | OH-O; O-O         |
|          | SER-4  | GLN-449              | 2.1; 2.8         | NH-O; N-O   | PHE-5  | PRO-479             | 3.6              | NH-O             |
|          | THR-7  | SER-452              | 3.2              | NH-O        | SER-4  | CYS-480             | 3.8              | O-NH             |
|          |        |                      |                  | SER-4      | ASN-481 | 3.5                |                 | OH-O             |
|          |        |                      |                  | TYR-2      | ASN-481 | 2.0; 3              | NH-O; O-N        |                 |
|          |        |                      |                  | PHE-5      | PHE-486 | 2.9                | NH-O             |                 |
| Satpdb28899 | ASP-20 | VAL-429              | 3.9              | O-O        | ARG-1  | GLU-484             | --               | 2.2; 2.8; 3.4    | NH-N; 2NH-O       |
| LEU-19  | VAL-429 | 2.9; 3.4             | NH-O; O-N        | ASP-20    | ARG-466 | 1.7; 2-6;           | NH-O; N-O        |                 |
| SER-16  | THR-458 | 1.8; 2.6             | OH-O; O-O        | LEU-19    | ARG-466 | 3.6                | NH-O             |                 |
| THR-15  | THR-458 | 3.6                  | NH-O             | GLU-17    | TYR-351 | 2.1; 3              | OH-O; O-O        |                 |
| GLU-17  | SER-349 | 2.9                  | OH-O             | THR-15    | TYR-351 | 3.7                | OH-O             |                 |
| SER-16  | ARG-346 | 2.0; 2.6             | NH-O; N-O        | GLU-17    | ARG-466 | 2.0                | NH-O; N-O        |                 |

Abbreviations: GRP78, glucose-regulating protein 78; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
A study recently showed by virtual screening that there are molecules that can in silico inhibit the GRP78 protein and prevent virus binding. Interestingly, our results also suggest a compound capable of simultaneously inhibiting the binding sites that allow entry of SARS-CoV-2 via GRP78. The peptides proposed in this work targets 2 regions of the S protein (III and IV) and 1 of GRP78. This interaction could interfere with or competitively bind to the S protein $S$ sites of SARS-CoV and GRP78. These results suggest a novel inhibitor mechanism distinct from the anti-SARS-CoV peptide that could disrupt the virus-host cell interaction. We propose, therefore, to combine 2 or 3 of these peptides before or after infection with the virus.

Inhibition of ATPase activity of the GRP78 NBD\textsuperscript{52} is another potential inhibitory mechanism of SARS-CoV-2 infection. Studies have previously reported that after competitive inhibition at the ATP-binding site of GRP78, the SBD of the latter adopts a conformation having a low affinity for the substrates, thus blocking their transport.\textsuperscript{53} Binding of various inhibitors to the ATP-binding site established by crystallographic studies\textsuperscript{54} allows altering the function of GRP78 and, therefore, theoretically avoids infection with SARS-CoV-2.

Pretreatment of GRP78 with the small molecule EGCG inhibited the Ebola virus infection,\textsuperscript{51} what supports our results obtained where EGCG is suggested to have an inhibitory effect on GRP78 with high affinity (~10.5 kcal/mol).
Interestingly, a recent study showed by molecular docking that certain natural products (estrogen and phytoestrogens) could interfere with the attachment of SARS-CoV-2 to stressed cells. As for the natural products (EGCG, homoeriodictyol, isorhamnetin, and curcumin) selected in our study, they could also interfere with this attachment.

There are several proofs on the antiviral potential of herbal compounds.56 Pharmacological investigations have revealed many pharmacological activities for certain phytochemicals as the EGCG, and Curcumin. Reducing the charge and inhibiting the expression of viral core proteins are the main effects of polyphenols.57,58 These effects are of great interest for the development of synthetic drugs against SARS-CoV-2-specific polyphenols. The inhibitory effect of curcumin on SARS-CoV replication has been demonstrated by Wen et al.59 Likewise, the impact of curcumin on cells before or after a viral infection reach the infectivity of certain viruses.60 Recently, mooring studies revealed that curcumin could potentially inhibit ACE2 to remove COVID-19 entry in the cell (potential effect). These results and that shown in our work are proof that confirms the potential role of curcumin as a promising antiviral agent.

### Table 3. Interaction residues between targets (SARS-CoV-2 Spike, GRP78) and small molecules (EGCG, homoeriodictyol, isorhamnetin, and curcumin).

| INTERACTION SITES | SMALL MOLECULE |
|-------------------|----------------|
|                   | EGCG           | HOMOERIODICTYOL | ISORHAMNETIN | CURCUMIN |
| ATPase domain of GRP78 Docking score | –10.2 kcal/mol | –9.0 kcal/mol | –8.8 kcal/mol | –8.2 kcal/mol |
| Residues Gly-228, Phe-258, Gly-364, Ser-365, Ile-368, Asp-390 | Thr-38, Thr-39, Ile-61, Gly-228, Glu-249, Asp-224, Phe-258 | Thr-38, Thr-39, Ile-61, Glu-201, Asp-224, Phe-258 | Thr-38, Thr-39, Ile-61, Glu-201, Asp-224, Phe-258 | Thr-39, Ile-61, Glu-201, Asp-224, Phe-258, Gly-228 |
| GRP78 (Spike Binding Site) Docking score | –10.5 kcal/mol | –8.1 kcal/mol | –7.2 kcal/mol | –7.7 kcal/mol |
| Residues Ser-455, Ser-452, Glu-427, Ala-454, Gln-458 | Glu-427, Gly-430, Ser-448, Gln-458 | Glu-427, Gly-430, Ser-448, Gln-458 | Glu-427, Gly-430, Ser-448, Gln-458 | Glu-427, Ser-425, Ile-450, Thr-441 |
| Spike (GRP78 Binding Site) Docking score | –10.0 kcal/mol | –7.5 kcal/mol | –7.9 kcal/mol | –7.9 kcal/mol |
| Residues Asn-422, Asp-420, Tyr-421, Lys-417, Asn-460, Lys-464, Val-427 | Asn-420, Asp-420, Tyr-421, Lys-417 | Tyr-421, Lys-417, Pro-463, Tyr-505 | Asn-420, Asp-420, Tyr-421, Lys-417 |

Abbreviations: EGCG, epigallocatechin gallate; GRP78, glucose-regulating protein 78; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The table details the hydrogen and hydrophobic links between the 2 targets and the small molecules.
with the viral spike; this specificity increases the probability of the compounds selected in our study to rival with the Spike and GRP78, thus preventing the entry of SARS-CoV-2 and the resulting infection.

Conclusions

Drug repositioning is an effective and rapid strategy for providing therapeutic solutions to COVID-19. In silico approaches can be very useful in identifying new indications for approved drugs whose pharmacokinetic data are already known, allowing them to move rapidly to the final phases of clinical trials.

In this work, we identified 4 phytochemicals (polyphenols) that could potentially inhibit the interaction of SARS-CoV-2 Spike protein with GRP78, in addition to 5 peptides (satpdb18674, satpdb18446, satpdb12488, satpdb14438, and satpdb28899) that target simultaneously the Spike protein and its binding region in GRP78. The satpdb18674 and EGCG gave the best results for all of the targeted sites for the 2 proteins. As these results appear very promising, further bioassays are needed to confirm the inhibitory activity of these compounds against SARS-CoV-2 infection.

Author Contributions

LA: Conceptualization, Methodology, Writing original draft, Writing-review and Editing.
FG: Conceptualization, Methodology, Writing original draft, Writing-review and Editing.
MK: Methodology, Writing-review and editing.
N el H: Writing-review and editing.
R el J: Writing-review and editing.
J el H: Writing-review and editing.
BI: Writing-review and editing.
LB: Writing-review and editing.
AI: Supervisor.

ORCID iDs

Hakmi Mohammed https://orcid.org/0000-0002-1548-3792
Badreddine Lmimouni https://orcid.org/0000-0001-9163-4911

REFERENCES

1. Gallagher TM, Buchmeier MJ. Coronavirus spike proteins in viral entry and pathogenesis. Virology. 2001;279:371-374.
2. Liu X, Wang Y, Liu Y, Chen H, Hu Y. Response of bacterial and fungal soil communities to Chinese fir (Cunninghamia lanceolate) long-term monoculture plantations. Front Microbiol. 2020;11:181.
3. Du L, Tai W, Zhou Y, Jiang S. Vaccines for the prevention against the threat of MERS-CoV. Expert Rev Vaccines. 2016;15:1123-1134.
4. Zhou Y, Jiang S, Du L. Prospects for a MERS-CoV spike vaccine. Expert Rev Vaccines. 2018;17:677-686.
5. Elfiky AA, Mahdy SM, Elshemey WM. Quantitative structure-activity relationship and molecular docking revealed a potency of anti-hepatitis C virus drugs against human corona viruses. J Med Virol. 2017;89:1040-1047.
6. Ibrahim IM, Abdelmalek DH, Elfiky AA. GRP78: a cell’s response to stress. Life Sci. 2019;226:156-163.
7. Li F. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol. 2016;3:237-261.
8. Saghazadeh A, Rezaei N. Towards treatment planning of COVID-19: rationale and hypothesis for the use of multiple immunosuppressive agents: anti-antibodies, immunoglobulins, and corticosteroids. Int Immunopharmacol. 2020;84:106560.
9. Coutard B, Vallee C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 2020;176:104742.
10. Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main protease (3CLpro) structure: basis for design of anti-SARS drugs. Science. 2003;300:1763-1767.
11. Perlman S, Netland J. Coronavirus infection: post-SARS: update on replication and pathogenesis. Nat Rev Microbiol. 2009;7:439-450.
12. Chu H, Chan C-M, Zhang X, et al. Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. J Biol Chem. 2018;293:11709-11726.
13. Ibrahim IM, Abdelmaltik DH, Elshahat ME, Elfiky AA. COVID-19 spike-host cell receptor GRP78 binding site prediction. J Infect. 2020;80:554-562.
14. Yang J, Nune M, Zong Y, Zhou L, Liu Q. Close and allosteric opening of the polypeptide-binding site in a human Hsp70 chaperone BiP. Structure. 2015;23:2191-2203.
15. Lee AS. The ER chaperone and signaling regulator GRP78/BiP as a monitor of endoplasmic reticulum stress. Methods. 2005;35:373-381.
16. Quinones QJ, de Ridder GG, Pizzo SV. GRP78: a chaperone with diverse roles beyond the endoplasmic reticulum. Histois Pathol. 2008;23:1409-1416.

17. Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. Antiviral Res. 2009;11:229-236.

18. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypical GRP78 in the nucleus regulates autophagic and therapeutic targeting. Biochem J. 2011;434:181-188.

19. Li J, Lee AS. Stress induction of GRP78/BiP and its role in cancer. Curr Med. 2006;6:45-54.

20. Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer. 2014;14:263-276.

21. Koller C, Maddato L, Li K, McCray PB Jr, Horan S, Gallagher T. The endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. J Virol. 1999;72:3851-3858.

22. Chan JF, Lau SK, To KK, Cheng VC, Woo PC, Yuen KY. Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. Clin Microbiol Rev. 2015;28:465-522.

23. Peck KM, Burch CL, Heise MT, Baric RS. Coronavirus host range expansion and Middle East respiratory syndrome coronavirus emergence: biochemical mechanisms and evolutionary perspectives. Annu Rev Virol. 2015;2:95-117.

24. Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. J Virol. 2015;89:1954-1964.

25. Chan C-M, Chu H, Wang Y, et al. Carcinoembryonic antigen-related cell adhesion molecule 5 is an important surface attachment factor that facilitates entry of Middle East respiratory syndrome coronavirus. J Virol. 2016;90:9114-9127.

26. Earnest T, Ugg S, Wychowski C, Dubuisson J. Involvement of endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. J Virol. 1999;72:3851-3858.

27. Gonzalez-Gronow M, Selim MA, Papalas J, Pizzo SV. GRP78: a multifunctional receptor on the cell surface. Antiviral Res. 2009;11:229-236.

28. Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. J Virol. 2015;89:1954-1964.

29. Chan C-M, Chu H, Wang Y, et al. Carcinoembryonic antigen-related cell adhesion molecule 5 is an important surface attachment factor that facilitates entry of Middle East respiratory syndrome coronavirus. J Virol. 2016;90:9114-9127.

30. Earnest T, Ugg S, Wychowski C, Dubuisson J. Involvement of endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. J Virol. 1999;72:3851-3858.

31. Aguiar JA, Tremblay BJ-M, Mansfield MJ, et al. Gene expression and functional screening of the hepatitis C virus genome. J Virol. 2011;85:1016-1035.e1019.

32. Ziegler CGK, Allon SJ, Nyquist SK, et al. SARS-CoV-2 receptor ACE2 is an unrecognized viral receptor. J Virol. 2020;10:07.030742v2.abstract.

33. Palmeira A, Sousa E, Köseler A, et al. Preliminary virtual screening studies to identify potential SARS-CoV-2 inhibitors. J Biomol Struct Dyn. 2020;16:301-306.

34. Allam L, Lakhlili W, Tarhda Z, et al. Three-dimensional structure prediction of SARS-CoV-2 receptor ACE2 in situ, free and bound to its ligands. J Biomol Res. 2020;11:e0154862.

35. Allam L, Mukherjee S, Karmakar SP, et al. GRP78 is an important host factor for Japanese encephalitis virus entry and replication in mammalian cells. J Virol. 2017;91:e02274-16.

36. Fioravanti R, Celestino I, Costi R, et al. Effects of polyphenol compounds on influenza A virus replication and definition of their mechanism of action. Bioorg Med Chem. 2007;15:4087-4095.

37. Elfiky AA. Natural products may interfere with SARS-CoV-2 attachment to the host cell [published online ahead of print May 5, 2020]. J Pharm Bioallied Sci. 2020;12:56-66.

38. Mahony JS, Pizarro JC, Park HW. Probing the ATP site of GRP78 with nucleotide triphosphate analogs. PLoS ONE. 2016;11:e0154862.

39. Allam L, Kakhtili W, Tarhda Z, et al. Three-dimensional structural prediction of the human LMTK3 catalytic domain in DTYG-in conformation. J Biomol Res. 2017;6:2.

40. O’Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchinson GR. Open Babel: an open chemical toolbox. J Cheminform. 2011;3:33.

41. rotational potential agents against coronaviruses: a review. Virus Res. 2020;284:197989.

42. Zhao Y, Jiang Y, Wu P, et al. Coronavirus disease 2019 (COVID-19): a perspective from China. Radiology. 2020;296:E15-E25.

43. Hakemi M, Bourica EM, Kandoussi I, Harti JE, Ibrahimi A. Repurposing of known anti-virals as potential inhibitors for SARS-CoV-2 main protease using molecular docking analysis. Bioinformation. 2020;16:301-306.

44. Nain M, Mukherjee S, Karmakar SP, et al. GRP78 is an important host factor for Japanese encephalitis virus entry and replication in mammalian cells. J Virol. 2017;91:e02274-16.

45. Surtelle AC, Costaantino JA, et al. HSPA5 is an essential host factor for Ebola virus infection. Antiviral Res. 2014;109:171-174.

46. Ermakova SP, Kang BS, Choi BY, et al. (-)-Epigallocatechin gallate overcomes resistance to etoposide-induced cell death by targeting the molecular chaperone glucose-regulated protein 78. Cancer Res. 2006;66:9260-9269.

47. Gaurasunghe KSN, Mishra A, Mishra S. Glucose-regulated protein 78 substrate-binding domain alters its conformation upon EGCG inhibitor binding to nucleotide-binding domain: molecular dynamics studies. Sci Rep. 2018;8:1-11.

48. Macias AT, Williamson DS, Allen N, et al. Adenosine-derived inhibitors of 78 kDa glucose regulated protein (Grp78) ATPass: insights into isoform selectivity. J Med Chem. 2012;55:4034-4041.

49. Palmeira A, Sousa E, Köseler A, et al. Preliminary virtual screening studies to identify potential SARS-CoV-2 inhibitors. J Biomol Struct Dyn. doi:10.1080/07391102.2020.1761881.

50. Praditya D, Kirkhoff L, Brüning J, Rachmawati H, Steinemann J, Steinemann E. Anti-infective properties of the golden spice curcumin. Front Microbiol. 2019;10:912.

51. Adem S, Eyooglu V, Sarfraz I, Aslul A, Ali M. Identification of potent COVID-19 main protease (Mpro) inhibitors from natural polyphenols: an in silico strategy unveils a hope against CORONA [published online ahead of print March 23, 2020]. PloS One. doi:10.1094/prep202003.0333.x1.

52. Clark K, Grant P, Sarr A, et al. An in vitro study of the flavaviruses extracted from black tea to neutralize bovine rotavirus and bovine coronavirus infections. Vet Microbiol. 1998;63:147-157.

53. Wen CC, Kuo YH, Jan JT, et al. Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. J Med. 2007;50:4087-4095.

54. Chen TY, Chen DY, Wen HW, et al. Inhibition of enveloped viruses infectivity by curcumin. PLoS ONE. 2013;8:e62482.

55. Triantafiloiu K, Fradelizi D, Wilson K, Triantafiloiu M. GRP78, a co-receptor for coxsackievirus A9, interacts with major histocompatibility complex class I molecules which mediate virus internalization. J Virol. 2002;76:633-643.

56. Wu Y-P, Chang C-M, Hung C-Y, Tsai M-C, Schuyler SC, Wang RY-L. Japanese encephalitis virus co-opts the ER stress response protein GRP78 for viral infectivity. J Biol Chem. 2011;8:1-10.