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A small molecule compound berberine as an orally active therapeutic candidate against COVID-19 and SARS: A computational and mechanistic study

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Abstract

The novel coronavirus disease, COVID-19, has grown into a global pandemic and a major public health threat since its breakout in December 2019. To date, no specific therapeutic drug or vaccine for treating COVID-19 and SARS has been FDA approved. Previous studies suggest that berberine, an isoquinoline alkaloid, has shown various biological activities that may help against COVID-19 and SARS, including antiviral, anti-allergy and inflammation, hepatoprotection against drug- and infection-induced liver injury, as well as reducing oxidative stress. In particular, berberine has a wide range of antiviral activities such as anti-influenza, anti-hepatitis C, anti-cytomegalovirus, and anti-alphavirus. As an ingredient recommended in guidelines issued by the China National Health Commission for COVID-19 to be combined with other therapy, berberine is a promising orally administered therapeutic candidate against SARS-CoV and SARS-CoV-2. The current study comprehensively evaluates the potential therapeutic mechanisms of berberine in preventing and treating COVID-19 and SARS using computational modeling, including target mining, gene ontology enrichment, pathway analyses, protein-protein interaction analysis, and

Abbreviations: 3CLpro3, chymotrypsin-like protease; ACE2, angiotensin-converting enzyme 2; AGE-RAGE, advanced glycation endproducts-receptor for advanced glycation endproducts; Akt, AKT serine/threonine kinase; BAX, BCL2 associated X, apoptosis regulator; BCL2, BCL2 apoptosis regulator; BCL2L1, BCL2 Like 1; BID, BH3 interacting domain death agonist; CASP, critical assessment of structure prediction; CCL2, C-C motif chemokine ligand 2; CCND1, cyclin D1; CDK4, cyclin-dependent kinase 4; CDKN1A, cyclin-dependent kinase inhibitor 1A; CHUK, component of inhibitor of nuclear factor kappa B kinase complex; CoV, coronavirus; COVID-19, coronavirus disease 2019; COX-2, cyclooxygenase-2; C-T-P-D, compound-target-pathway-disease; CXCL2, C-X-C motif chemokine ligand 2; EGFR, epidermal growth Factor Receptor; ERK, extracellular signal-regulated kinase; FDR, false discovery rate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GO, gene ontology; IFN, interferon; IKBA, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IL, interleukin; JUN, Jun proto-oncogene, AP-1 transcription factor subunit; MAPKs, mitogen-activated protein kinases; MERS-CoV, middle east respiratory syndrome-related coronavirus; MMP, matrix metallopeptidase; MYC, MYC proto-oncogene, BHLH transcription factor; NADPH, nicotinamide adenine dinucleotide phosphate; NFκB1, nuclear factor kappa B subunit 1; NFκB1A, NF-κB inhibitor alpha; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NOD-like receptor family pyrin domain containing 3; NOD, nucleotide binding oligomerization domain; PGE2, prostaglandin E2; Plpro, papain-like protease; PPI, protein-protein interaction; PTGS2, prostaglandin-endoperoxide synthase 2; RdRp, RNA-dependent RNA polymerase; ROS, reactive oxygen species; SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TCM, traditional Chinese medicine; TMPRSS2, transmembrane serine protease 2; TNF, tumor necrosis factor; TP53, tumor protein P53; VEGFA, vascular endothelial growth factor A.

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in silico molecular docking. An orally available immunotherapeutic-berberine nanomedicine, named NIT-X, has been developed by our group and has shown significantly increased oral bioavailability of berberine, increased IFN-γ production by CD8+ T cells, and inhibition of mast cell histamine release in vivo, suggesting a protective immune response. We further validated the inhibition of replication of SARS-CoV-2 in lung epithelial cells line in vitro *(Calu3 cells)* by berberine. Moreover, the expression of targets including ACE2, TMPRSS2, IL-1α, IL-8, IL-6, and CCL-2 in SARS-CoV-2 infected Calu3 cells were significantly suppressed by NIT-X. By supporting protective immunity while inhibiting pro-inflammatory cytokines; inhibiting viral infection and replication; inducing apoptosis; and protecting against tissue damage, berberine is a promising candidate in preventing and treating COVID-19 and SARS. Given the high oral bioavailability and safety of berberine nanomedicine, the current study may lead to the development of berberine as an orally, active therapeutic against COVID-19 and SARS.

KEYWORDS
anti-viral, apoptosis, berberine, computational modeling, COVID-19 and SARS

1 | INTRODUCTION

Coronavirus disease-19 (COVID-19) is an infectious disease caused by a newly discovered coronavirus SARS-CoV2 that has reached global pandemic status and become a major global health threat. As of December 1 2020, there have been 63,751,931 confirmed cases and 1,477,976 deaths worldwide.[1] The United States hit record-high daily COVID-19 cases in November, 2020. Since the pandemic started in March, 2020, the nation has surpassed 12 million cases and more than 266,000 Americans have died. United States could see "a surge upon a surge" of COVID-19 cases this winter.[2] During just two full days at the end of November, 2020, the country saw over 360,000 new COVID-19 cases nationwide, in addition to over 2,700 new deaths. In 2003, a zoonotic coronavirus outbreak of SARS-CoV had resulted in severe SARS with fatality rates of 10%.[3-6] The SARS-CoV-2 genome shares approximately 70%-80% sequence similarity to SARS-CoV, and causes similar clinical symptoms.[7,8] Key clinical features of COVID-19 and SARS include fever, chills, muscle pain, headache, sore throat, new loss of taste or smell, cough, shortness of breath, gastrointestinal problems in mild to moderate cases, and more serious disease involving pneumonia, acute respiratory distress syndrome, cardiovascular and hepatic failure with high morbidity.[7,8] Individuals with pre-existing conditions like cardiovascular disease, hypertension, asthma, and diabetes,[9] and elderly patients are at a higher risk to become infected with severe symptoms.[10] To date, no specific therapeutic drug or vaccine COVID-19 and SARS is available, resulting in an urgent need for broad-spectrum therapeutics for COVID-19 and other CoV infections.

Traditional Chinese medicine (TCM) has been highly recommended by the government of China to treat COVID-19 patients.[11] Natural products from TCM remain a rich source for the development of novel therapeutic agents for the treatment of COVID-19 and SARS. Berberine, a natural isoquinoline alkaloid, is a medicinally valuable natural compound with published anti-inflammatory, antiviral, antibacterial, anticancer, and antiparasitic activities, and having beneficial effects in hypertension, diabetes, and neurodegenerative conditions[12-14] Existing literature suggests that berberine has protective effects against drug-[15] or infection-induced[16] liver, heart, and neuronal cell damage.[12-14] Moreover, berberine has shown a wide range of antiviral activities[17] such as anti-influenza,[18] anti-hepatitis C,[19] anti-cytomegalovirus,[20] and anti-alphaviruses.[21] Berberine is also an ingredient recommended in guidelines issued by China National Health Commission for COVID-19 to be combined with other therapies.[8] In 2014, we discovered the IgE-lowering property of berberine,[22,23] showing excellent efficacy in treating allergic diseases. Moreover, to overcome berberine's low biovaliability,[24,25] we developed an oral immunotherapeutic berberine nanomedicine (NIT-X), for treating food allergy.[26] Our previous studies showed that a once-a-day oral NIT-X (2 mg/mouse) for 4-weeks resulted in 98%-100% reduction in IgE and 100% protection against anaphylaxis in peanut-allergic mice, which is associated with suppressing histamine release by mast cells, and induction of IFN-γ by CD8+ T cells (Srivastava et al manuscript in preparation, 2020).

Pro-inflammatory cytokines like IL-6, IL-1α/β, TNF-α, IL-8, and MCP-1 (CCL2) promote severity of disease and tissue damage in COVID-19.[27] The major cause of mortality

is associated with hyper-inflammation resulting in a cytokine storm. Moreover, recent meta-analysis showed that a high IL-6/IFN-γ ratio was associated with severe COVID-19 cases.[28] SARS-CoV-2 infection may primarily affect T lymphocytes, particularly CD4+ and CD8+ T cells, resulting in decreased IFN-γ production[29] and adding IFN-γ to type I IFNs as a synergistic combination therapy for COVID-19 has been suggested.[30] Chronic inflammation and allergy share several molecular targets with COVID-19. For example, the NFκB signaling pathway—one of two signals required for allergy-prone IgE production[31]—and NFκB driven inflammatory cytokine production (TNF-a, IL-6, IL-1β, IL-8, etc) are involved in recalcitrant asthma.[32,33] Impaired IFN-γ leads to susceptibility to hyperreactivity[34] and was associated with severe COVID-19 cases in young people.[35] In addition, mast cell activation, the key mechanism involved in anaphylaxis, has been suggested to be involved in hyper-inflammation in COVID-19 patients and anti-IgE and mast cell mediator antagonists appear to be helpful for COVID-19 patients.[36-39]

Early response of IFNs is critical for combatting SARS-CoV-2.[29,30] Berberine has been shown to increase the production of INF-γ, and inhibit Th2 responses,[40] indicating its potential role in protective immunity for infectious diseases.[41] Previously, we also found that in vivo treatment with berberine/NIT-X resulted in significant elevation of IFN-γ and CD8+ T cells in peanut-restimulated splenocytes in a murine model. Furthermore, berberine was shown to mitigate inflammatory cytokine production by downregulation of MAP Kinase and ERK and downregulation of pro-inflammatory transcription factors NFκB and AP-1.[12,42-44] The hallmarks of SARS and COVID-19 disease are unchecked viral replication and serious multiorgan tissue damage.[45-48] Berberine/NIT-X might alleviate tissue damage by reducing inflammation-induced death signals (RAGE, NLRP3/Caspase).[49,50] With excellent efficacy in treating allergy, we hypothesized that berberine/NIT-X would likewise be effective against COVID-19 and SARS by supporting protective immunity[41] while inhibiting pro-inflammatory cytokines and viral replication, inducing apoptosis, and protecting against tissue damage.

The current study seeks to evaluate the potential therapeutic mechanisms underlying the efficacy of berberine/NIT-X in preventing and treating COVID-19 and SARS using computational modeling—target mining, gene ontology enrichment, pathway, and protein-protein interaction analyses, and in silico molecular docking. The entire workflow of the study is shown in Figure 1. In addition, the inhibition of replication of SARS-CoV-2 by berberine/NIT-X has been validated in vitro. The suppression of ACE2, TMPRSS2, IL-1α, IL-8, IL-6, and CCL-2 by berberine/NIT-X were further validated. The results of these analyses will contribute to a molecular-level understanding of how berberine functions in the prevention and treatment of COVID-19 and SARS, providing both the rationale and tools needed to further validate its efficacy. Given the discovery of the high oral bioavailable ability of berberine nanomedicine (NIT-X),[26] the current study may lead to a development of berberine/NIT-X as an orally active therapeutic against COVID-19 and SARS.

2 | MATERIALS AND METHODS

2.1 | Target mining

Biological targets of berberine were identified from literature reports[51,52] and published databases including Similarity Ensemble Approach,[56,57] PubChem,[58,59] and DrugBank.[60,61] The relevant human genes associated with COVID-19 and SARS were selected as drug targets from various databases including Therapeutic Target Database,[62,63] Genetic Association Database,[64,65] GeneCards,[66,67] Open Targets Platform,[68,69] and Comparative Toxicogenomics Database.[70,71] The lung specific targets were collected from Open Targets Platform[68,69] and literature.[72] To ensure the predominance of targets, only the top 100 genes in each database were considered. Selected targets were finally mapped to UniProt Database[73,74] for normalization. Next, the shared targets of berberine with COVID-19 and SARS were obtained and these were considered to be potential regulated targets of berberine for the prevention and treatment of COVID-19 and SARS.

2.2 | Gene ontology (GO), pathway, and protein-protein interaction (PPI) analysis

Target enrichment gene ontology, pathway, and protein-protein interaction (PPI) analyses provided a molecular-level mechanistic insight into biological function. GO was introduced by mapping potential targets to the DAVID database.[75,76] The GO biological process terms with a false discovery rate of (FDR) < 0.01 were selected. Pathways were obtained by mapping targets to KOBAS 3.0[77,78] and the significant pathways with FDR < 0.01 were selected. Potential targets were mapped to String database, obtaining their interaction. The protein interactions were further used to construct the PPI network using Cytoscape (v3.2.1).

2.3 | Compound-target-pathway-disease (C-T-P-D) network construction and analysis

With obtained targets and significant pathways, C-T-P-D biological networks were constructed using Cytoscape (v3.2.1). The C-T-P-D network, containing berberine, its related targets for COVID-19 and SARS, and significant principal pathways provided general information about pharmacological

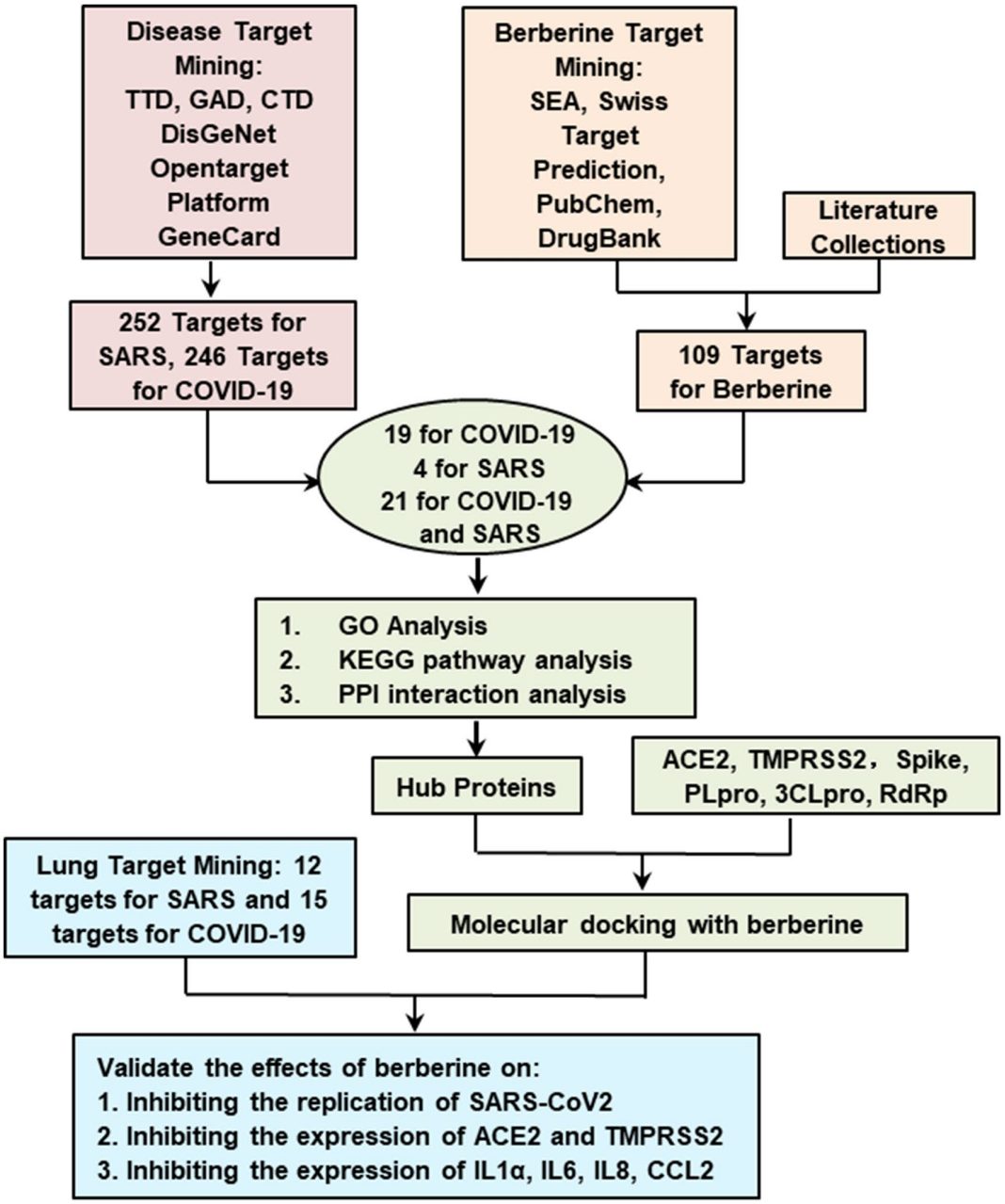

FIGURE 1 The workflow of computational modeling used for analysis of berberine as a promising candidate against COVID-19 and SARS. TTD: therapeutic target database; GAD: genetic association database; SEA: similarity ensemble approach; PPI: protein-protein interactions; ACE2: angiotensin-converting enzyme 2; TMPRSS2: transmembrane serine protease 2; PLpro: Papain-like Protease; 3CLpro: coronavirus main proteinase; RdRp: RNA-dependent RNA polymerase. First, a total of 254 genes for SARS and 247 genes for COVID-19 were selected as possible targets for berberine. Separately, 109 inflammatory and biological targets of berberine were collected based on literatures and following published databases: hitpick, swiss target prediction, SEA, pubchem, and drugbank. Mining berberine targets onto identified disease targets uncovers that berberine might potentially regulate 21 targets for both COVID-19 and SARS, 4 targets for SARS specifically, and 19 targets for COVID-19 specifically. With biological targets of berberine for the prevention and treatment of COVID-19 and SARS established, GO, KEGG pathway, and PPI analysis were conducted to uncover the mechanistic details of berberine regulation in key pathways. Moreover, hub host proteins are determined for further molecular docking analysis. Combining crucial virus proteins and vital host receptor proteins in virus infection and duplication process, molecular docking was further applied to investigate the possible binding modes of berberine to these targets. Based on the lung target mining and molecular docking results, the inhibition of berberine on ACE2, TMPRSS2, IL1α, IL6, IL8, and CCL2 were further validated

mechanisms of berberine for the prevention and treatment of COVID-19 and SARS at a molecular level. The properties of C-T-P-D networks were validated by NetworkAnalyzer,[79] a plugin of Cytoscape.

2.4 | Molecular docking analysis

The hub 10 proteins in C-T-P-D network were selected as highly promising targets of berberine. Moreover, human

receptors and virus proteins involved in viral infection and replication processes were all considered as potential targets of berberine. Molecular docking was performed on berberine with obtained targets by AutoDock Vina[80] to further explore their binding modes. Protein crystal structures including NFκB1 (PDB:1NFK), CHUK (PDB:5EBZ),[81] MAPK3 (PDB:4QTB),[82] MAPK1 (PDB:4O6E),[83] CASP3 (PDB:1PAU), IL6 (PDB: 1ALU),[84] MAPK8 (PDB:2H96),[85] BAX (PDB:4S0O),[86] and TNF (PDB:2AZ5),[87] ACE2 (PDB:1R4L),[88] 3CLpro (PDB:6LU7),[89] Spike (PDB: 6VW1), PLpro (PDB: 6W9C), and RdRp (PDB:6M71)[90] with excellent resolution were downloaded from RCSB protein data bank (www.rcsb.org/).[91] Protein structures of NFκB1A were built by homology modeling.[92] Structure of TMPRSS2 was obtained from a reported model.[93] The structure of berberine was directly downloaded from PubChem (pubchem.ncbi.nlm.nih.gov/)[59,94] without further optimization. Proteins and berberine were prepared by AutoDockTools (v1.5.6).[95] The molecular graphics were prepared by PyMOL system[96] (http://www.pymol.org) and Discovery Studio.[97] Generally, all hydrogens and Gasteriger charges were added to each molecule. Docking areas and AutoGrid parameters were set based on the binding pockets of proteins.

2.5 | Cell culture

Human epithelial cells, Calu-3 (American Type Culture Collection; Rockville, MD), were used to evaluate the effect of NIT-X on SARS-CoV-2 viral replication, ACE2, TMPSS2, and cytokine and chemokine expression. Cells were cultured at 37°C under 5% CO_2 in complete EMEM medium supplemented with 20% FBS, and 1% penicillin-streptomycin. Cells were seeded at an initial concentration of 5×10^5 cells/mL and medium was changed every 3 days.

2.6 | Cell infection and real time polymerase chain reaction (PCR)

Calu-3 cells were seeded in six well plates with 5×10^5 cells per well. After 24 hours, cells were incubated in media containing 20 μg/mL, 40 μg/mL berberine/NIT-X, or DMSO (v/v 1:1000). After 3 days incubation, cells were infected with SARS-CoV-2 (MOI = 0.005) or mock (-) for 1 hour and grown in indicated media for 24 hours. At 1-day post infection (1 dpi), cells were harvested and total RNA in mock or infected cells were extracted using Trizol. A 200 ng of total RNA was used for cDNA synthesis. Real Time-PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) with SARS-Cov-2 Nucleocapsid (N) proteins, ACE, TMPSS2, IL-6, IL-8, IL-1α, CCL2, and HPRT primers. The primer sequences are listed as in Table S1. Data were normalized to HPRT and presented as 2-ΔCT.

2.7 | Cell viability assay

Cell viability by CCK-8 assay of berberine/NIT-X on Calu3 cells was performed using a commercial kit (Dojindo, Rockville, MD). A 6.5×10^3/well Calu3 cells were seeded on 96 well plate and preincubated for 24 hours. Next, berberine/NIT-X at 20 μg/mL and 40 μg/mL were added in corresponding wells and cells were incubated for another 24 hours. A 10 μL of CCK-8 solution was then added into each well and incubated for 1 hour. The plate was read at 450 nm. Cell viability was analyzed by comparing the OD value.

3 | RESULTS AND DISCUSSION

3.1 | Target mining identifies the shared biological targets between berberine, COVID-19, and SARS

The Venn diagram in Figure 2 shows that 252 genes for SARS and 246 genes for COVID-19 were selected, totaling 336 genes. Of these, 155 genes were shared between COVID-19 and SARS, supporting the rationale to develop therapies for both diseases. Moreover, 109 biological targets of berberine were collected from the literature,[51,52] and published databases. Among them, 21 shared targets for berberine with both COVID-19 and SARS; 19 shared targets for berberine and COVID-19; and four shared targets for berberine and SARS were discovered, yielding 44 shared targets between berberine and COVID-19 and SARS, which were finally selected as the main targets of berberine in preventing and treating COVID-19 and SARS.

3.2 | Gene ontology (GO) reveals potential regulation of berberine in apoptosis, proliferation, and host systematic reactions

GO biological process terms in DAVID database were obtained with the identified targets as an enriched gene-set. The top 15 biological process GO terms with FDR < 0.01 were ranked by enrichment score (-logFDR) in Figure 3A. The host acts against pathogenic microbes by inducing both innate and adaptive immune responses.[98] Most significant GO biological process terms are closely associated with the change in states or activities of cells encountering viral infection, such as proliferation, differentiation, secretion, gene expression, and apoptosis. Among them, inducing apoptosis in host cells at early-stage infection by viruses has been considered as a

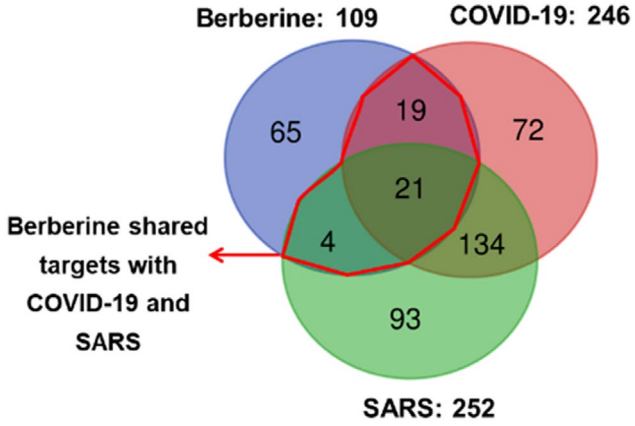

FIGURE 2 Venn diagram showing shared targets between berberine, COVID-19, and SARS. Among them, 21 shared targets are identified for berberine with both COVID-19 and SARS, 19 shared targets for berberine and COVID, and 4 shared targets for berberine and SARS

self-defense mechanism.[99,100] In infected cells, the viruses are likely to be destroyed along with phagocytized and digested processes of apoptotic cells. Cell apoptosis has been observed in a wide range of human viral infections,[101] for instance, cardiocyte apoptosis during both active and chronic viral myocarditis,[102] hepatocyte apoptosis during hepatitis B virus[103] and hepatitis C virus infections,[104] and apoptosis during influenza virus infection.[105] Berberine has been demonstrated to induce biphasic cell death in treating hepatitis C virus-induced hepatocellular carcinoma—first triggering apoptosis in early-stage at 24 hours post-berberine treatment that then progressing to necrotic cell death at 48 hours posttreatment.[106] Apoptosis induced by berberine through mitochondria/caspases pathway in cancer cells has been widely investigated,[107-109] which may be a vital regulated process of berberine to prevent viral proliferation in early infection. Based on GO analysis results, regulated apoptosis by berberine includes "negative regulation of apoptotic process," "apoptotic process," "extrinsic apoptotic signaling pathway in absence of ligand," "activation of cysteine-type endopeptidase activity involved in apoptotic process," and "regulation of apoptotic process." In addition, most viral infections including SARS-CoV infection may induce a transient state of immune suppression and cell proliferation inhibition.[110,111] Cell proliferation, especially immune cell proliferation, plays a crucial role in combatting viral infection. However, in hyperinflammation caused by COVID-19 and SARS, immunosuppression is likely to be beneficial.[112] Berberine has been reported to cause G0/G1[113] and G2/M[114,115] cell arrest leading to inhibition of cell proliferation in different cell lines. The proliferation-related process, including "positive regulation of cell proliferation" and "cell proliferation," displays the potential efficacy of berberine in regulating cell proliferation and balancing the immune response to resist COVID-19 and SARS. Moreover, the comprehensive cell activities include "response to drug," "cellular response to organic cyclic compound," "response to estradiol," "cellular response to mechanical stimulus," "response to toxic substance," and "cellular response to DNA damage stimulus," standing for systemic regulation of berberine for host systematic reaction after a viral stimulus.

3.3 | Pathway analysis reveals complex signal transduction regulated by berberine

Regulated Kegg pathways were obtained with these identified targets as an enriched gene-set. The top 15 significant pathways with FDR < 0.01 were ranked by enrichment score (-log-FDR) in Figure 3B. The involved genes in each pathway are listed in Figure S1. The pathway results are consistent with the main points deduced from GO analysis. Most pathways (53%) are related to host immune responses to viral infection including "Human cytomegalovirus infection (P2)," "Hepatitis B (P3)," "Kaposi sarcoma-associated herpesvirus infection (P4)," "Measles (P6)," "Epstein-Barr virus infection (P8)," "Hepatitis C (P9)," "Influenza A (P10)," and "Human T-cell leukemia virus 1 infection (P14)." During viral infection, both innate and adaptive immune reactions are activated to regulate various cell activities through signaling transduction.[98] Moreover, "NOD-like receptor signaling pathway (P11)" is an immune-related pathway, which is responsible for detecting various pathogens and generating innate immune response.[116] Especially, NLRP3 activation is well recognized as a trigger for CoV inflammatory cascade and tissue damage.[117-119] Berberine has been reported to alleviate influenza virus-induced inflammatory lesions by restricting NLRP3 inflammasome activation through decreasing ROS generation.[49] NLRP3 may, therefore, be a high priority target of berberine against CoVs in the regulation of "NOD-like receptor signaling pathway (P11)." "Apoptosis (P5)" further emphasizes the regulation of apoptosis by berberine, which is an innate immune response to viral infection. The cytokine-related pathways include "IL-17 signaling pathway (P12)" and "TNF signaling pathway (P13)," which play a crucial role in modulating immune pathophysiology of viral infection.[120,121] It is reported that the severity of COVID-19 and SARS positively correlates with levels of Th17 cell-related pro-inflammatory cytokines including IL-17, IL-6, IL-1, TNF, and IFN-γ.[122] And berberine has been found to regulate differentiation and amelioration of Th1 and Th2 cell to impact the corresponding cytokines,[123] indicating IL-17 and TNF might be the main cytokines regulated by berberine to control the course of infection. In addition, the "AGE-RAGE signaling pathway in diabetic complications (P7)" is related to inflammatory regulation. AGE-RAGE signaling elicits activation of multiple intracellular signaling pathways involving NADPH oxidase, protein kinase C, and MAPKs, then, resulting in NF-κB activation.[124] Much evidence[125] shows that pulmonary tissues express a remarkably

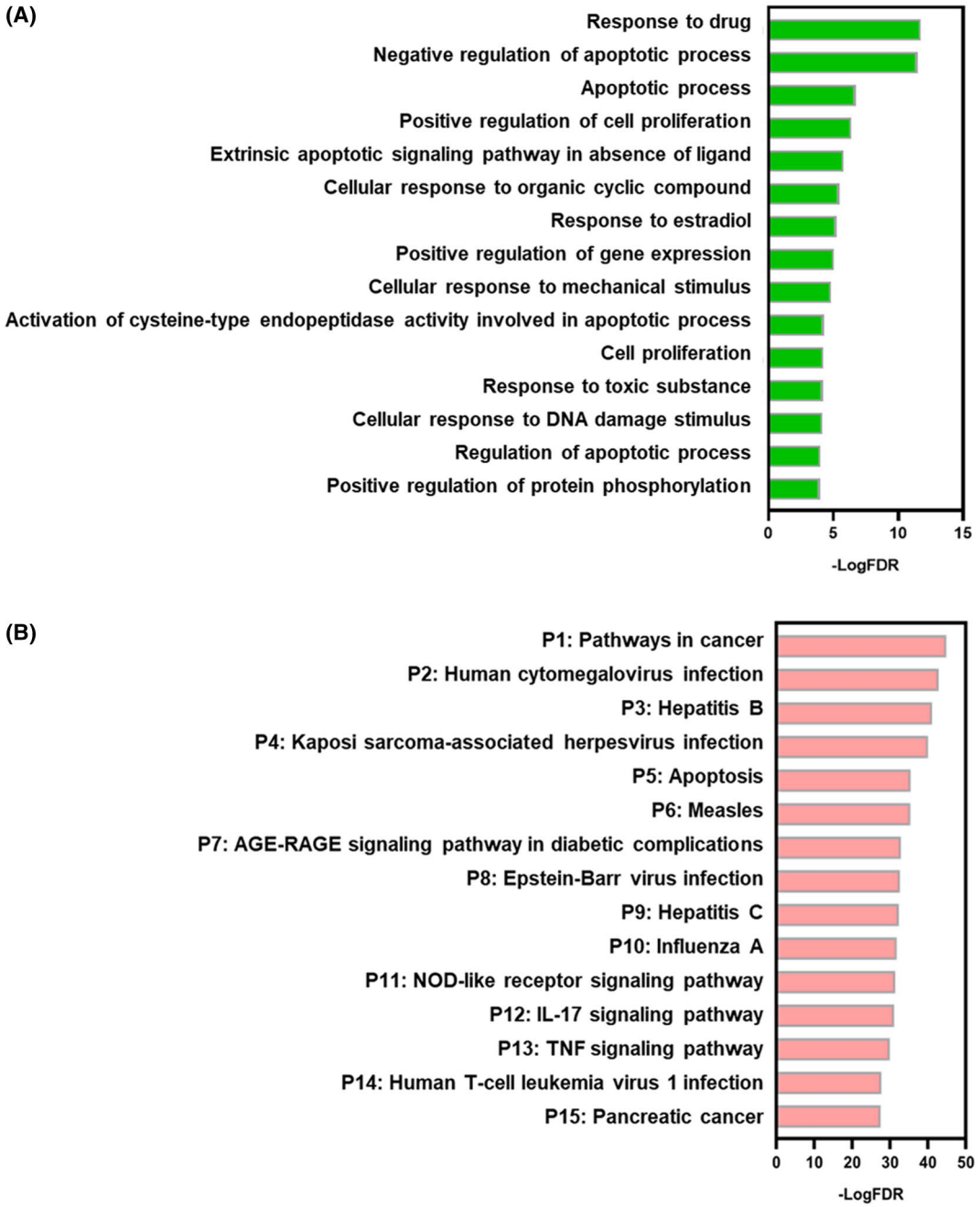

FIGURE 3 Gene ontology (GO) biological process (BP) analyses and pathway analyses of the targets. A, GO BP analyses; B, Pathway analysis; *Y*-axis: top 15 biological processes (A) and top 15 pathway (B) relevant to the enriched targets; *X*-axis: significance of each term ranked with –log (false discovery rate) (FDR)

high basal level of RAGE, which is a key molecule in the onset and sustainment of the inflammatory response in many disease pathologies. Berberine has been discovered to regulate AGEs-RAGE signaling pathway in mesangial cells exerting renoprotective effects during diabetic nephropathy,[50] which might indicate its possible role in regulation of AGEs-RAGE signaling during SARS-CoV and SARS-CoV-2 infection.[126] In addition, "Pathway in cancer (P1)" and "Pancreatic cancer (P15)" stand for complex pathways involving various activities including inflammatory process, metabolic regulation, cell proliferation, and cell apoptosis, part of which may be regulated by berberine to resist against the virus.

3.4 | Compound-target-pathway-disease (C-T-P-D) **network construction to select the crucial proteins**

C-T-P-D network (Figure 4) containing berberine, selected targets, top 15 pathways, and COVID-19 and SARS as

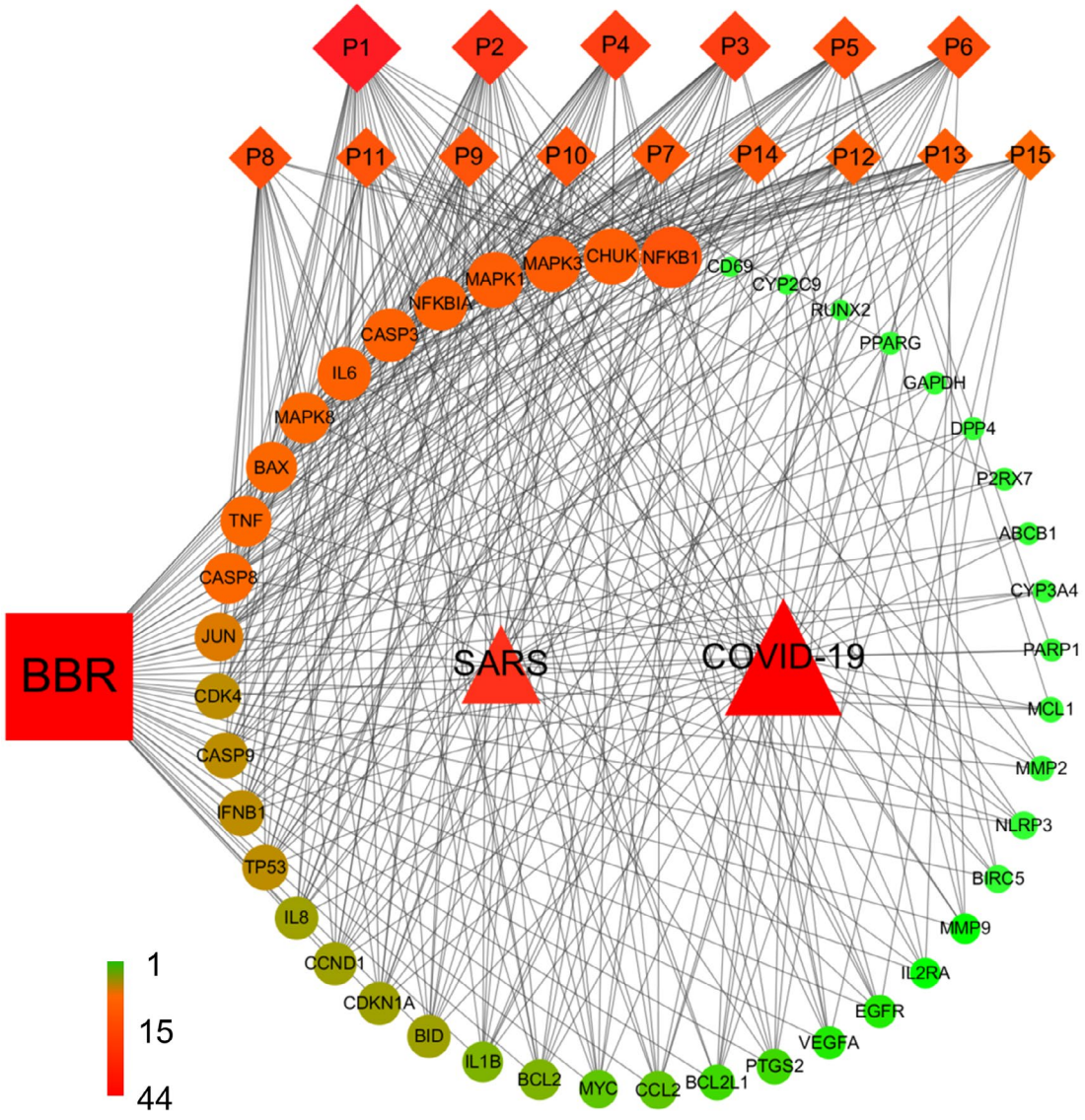

FIGURE 4 Compound-Target-Pathway-Disease (C-T-P-D) network of the berberine for COVID-19 and SARS treatment. Squares, circles, diamonds, and triangles represent berberine, common targets, pathways and diseases, respectively. Node size and node color (ie, from green (lowest) to red (highest) indicate a measure of degree. Black lines represent interaction between nodes

diseases, provides general information about the potential pharmacological mechanisms of berberine for prevention and treatment of COVID and SARS at the molecular level. We believe that the frequency of targets appearing in the top 15 pathways imply their influence and importance. Node sizes from large to small, and color from red to green, are proportional to degree value, displaying their importance from high to low in the network. The encoded proteins of NFκB1 (p50 and p105), CHUK (IKK), NFκB1A (IKBA), and TNF (TNF-α) all play crucial roles in NF-κB signaling pathway, which participates in multiple aspects of innate and adaptive immune functions and serves as a pivotal mediator of inflammatory responses.[127] The activation of NF-κB can not only induce the expression of various pro-inflammatory cytokines and chemokines[127,128] such as TNF-*α*, IL-8, IL-6, IL-1β, and COX-2, but also regulate the survival, activation and differentiation of innate immune cells and inflammatory T cells,[129,130] causing hyper-inflammation and severe tissue damage during viral infection. It has been demonstrated that treatment with NF-κB inhibitors did not affect virus titers but reduced expression of cytokines such as TNF, CCL2, and CXCL2; alleviated lung pathology in both SARS-CoV-infected cultured cells and mice; and significantly increased mouse survival.[131] In addition, NF-κB signaling participated in most of the top 15 pathways, further indicating its pivotal role. Berberine has been found to suppress the activation of NF-κB through inhibition of various inflammatory agents, such as direct inhibition of IκB kinase (IKK) activation and inhibition of PPARγ activation, which may contribute in part to the inhibition of NF-κB.[132] Thus, proteins in NF-κB signaling transduction

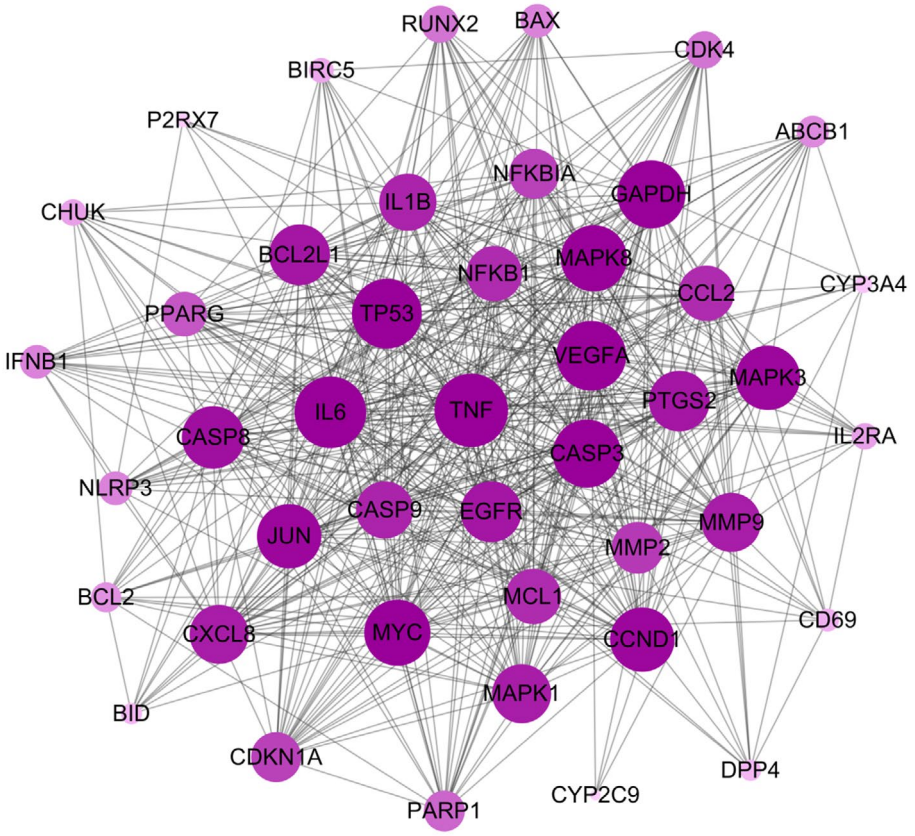

FIGURE 5 Protein-protein interactions. Circles represent the targets. Black lines represent the interaction between nodes. Node size is proportional to its degree in network

might be promising main targets of berberine to suppress the hyper-inflammation during SARS-CoV and SARS-CoV2 infection. Similarly, MAPKs are members of all top 15 pathways. MAPK3, MAPK1, and MAPK8, signal transducers responding to extracellular stimulation by cytokines, growth factors, viral infection, and stress can regulate cell differentiation, proliferation, survival, and apoptosis.[133-135] It is reported that SARS-CoV causes the activation of physiological intracellular signaling cascades leading to the phosphorylation and activation of p38 MAPK signaling pathway.[135] Moreover, a subset of the licensed kinase inhibitors targeting the ERK/MAPK pathway has been demonstrated to significantly inhibit MERS-CoV propagation in vitro regardless of whether they were added before, or after viral infection.[101] Downregulated expression of MAPKs was detected in recovered COVID-19 patients, indicating the MAPK signal pathway may be one sign of patient recovery.[136] Berberine has been found to hamper influenza A and enterovirus 71 replication through inhibition of MAPK/ERK signaling pathway,[137,138] thus, inhibition of the ERK signaling pathway is one of the primary potential mechanisms of action for berberine suppression of SARS-CoV and SARS-CoV-2 replication.[139] Apoptosis-related genes including CASP3, CASP8, BAX, BID, BCL2, and BCL2L1 can initiate apoptotic signaling (P5) via the extrinsic pathway or intrinsic pathway. The importance of apoptotic regulation for COVID-19 and SARS treatment has been discussed in both GO and pathway analysis. CASP3, CASP8, and BAX are known targets of berberine in regulating apoptosis[109,140,141] and may in fact be the critical targets in inducing apoptosis of cells infected by SARS-CoV and SARS-CoV-2. Moreover, the inhibition of pro-inflammatory cytokines (eg, TNF-*α,* IL-1α/β, and IL-6) by berberine would prevent infected cells from pyroptosis and reduce the tissue damage in late stages. Cytokines and chemokines, including IL-6, TNF-*α,* IFN-β, IL-8, IL-1α/β, CCL2, MMP9, and MMP2, have all been confirmed to be closely associated with pathogenesis of COVID-19 and SARS.[142-144] Among them, it has been reported that the high levels of IL-6 are activated by the viral nucleocapsid SARS-CoV N protein, causing lung lesions in SARS patients.[145] The increased expression of IL-6 and IL-8 in serum is expected to predict the severity of COVID-19 pneumonia and the prognosis of patients in the clinic[146] and IL-1α/β could mediate the inflammation of the lungs, fever, and fibrosis thus causing respiratory complications in the infected host.[146] Berberine has been shown to significantly suppress TNF-α and IL-6 expression induced by HIV protease inhibitors even at low concentrations.[147] The production of IL-1α/β and TNF-α have been reported to be suppressed by berberine via the inhibition of IκB degradation in human lung cells.[44,148] Berberine can regulate the production or effects

of inflammatory cytokines directly or indirectly,[149,150] which make it a promising agent in the prevention and treatment of COVID-19 and SARS. Moreover, the regulation of cell proliferation by berberine has been mentioned previously. The targets related with cell proliferation and cell cycle include CDK4, TP53, CCND1, CDKN1A, and MYC and it is known that berberine can up-regulate varicocele-induced CDK4 and CCND1 expression reduction in rat testicles.[151] Berberine may also balance cell proliferation and apoptosis during viral infection via signaling regulation. In conclusion, these genes are all promising targets on which berberine may act to regulate immune responses, inflammatory processes, and cell activities against COVID-19 and SARS infection. The top 10 targets in the network were chosen for further molecular docking analysis.

3.5 | Protein-Protein interaction (PPI) network construction to confirm the vital function of proteins

The PPI network (Figure 5) was constructed by mapping potential targets to the String database.[152] The size of the node from large to small is proportional to its degree value in the network. It is well known that protein-protein interactions are critical to a wide range of biological processes, including cell-to-cell interaction and metabolic and developmental control.[153] Deeper understanding of such complex relationships among disease-related proteins provides new opportunities to investigate the molecular mechanisms of diseases.[154] Recently, PPI has become a reliable tool to evaluate protein functions in the network and determine hub proteins in the regulation of diseases. As expected, TNF (TNF-α) occupied the central position in the network, indicating its close associations with the other proteins. TNF-α belongs to the TNF superfamily of cytokines, which regulates dozens of pathways related to cell proliferation, differentiation, survival, and death.[155] Following, IL-6, NFκB1, CASPs, and MAPKs have previously all been selected as pivotal targets of berberine, and will not be discussed further here. As with the C-T-P-D network, proteins such as TP53 and JUN hold a moderate rank in the PPI network; however, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) seems to display more connections in the PPI network. Besides association with cellular glycolysis, GAPDH can modulate cellular signaling pathways in response to oxidative stress, and participates in cell death/dysfunction processes in the nucleus.[156] In particular, GAPDH has been demonstrated to bind with telomeres and protect telomeric DNA from rapid degradation,[157] with telomere length possibly playing an important role in maintaining the rapid replicative response of leukomonocytes in the face of SARS-CoV and SARS-CoV-2 infection.[158] Berberine has been proven to regulate the level of GAPDH in adipocyte differentiation,[159] indicating that berberine may affect telomere status through regulating GAPDH during SARS-CoV and SARS-CoV-2 infection. Moreover, it's worth noting that targets including VEGFA, CCND1, EGFR, and PTGS2 play a critical role in signal transmissions in PPI. Vascular Endothelial Growth Factor (VEGF) is considered to be the most potent vascular permeability inducer and recent evidence[77] has revealed higher VEGF levels in COVID-19 patients compared with healthy controls. The rise of VEGF levels may be caused by hypoxia, severe inflammation, and upregulation of the infected respiratory tract epithelium itself.[160,161] CCND1 is related to cell proliferation and apoptosis, and this has been fully discussed earlier. Activation of EFGR triggers the signaling of MAPK, Akt, and JNK pathways, resulting in a range of outcomes, such as inhibition of apoptosis, increase in cell proliferation and migration, activation of the inflammatory response, and increase of IL-8 production.[162] EFGR signaling has been shown to mediate pulmonary fibrosis in the hyperactive host response to SARS-CoV infection. And the inhibition of EGFR signaling may prevent an excessive fibrotic response to SARS-CoV and other respiratory viral infections.[163] It has been reported that berberine could suppress the constitutive activation of EGFR in tumor cells to inhibit growth and induce apoptosis.[164] Thus, EGFR may be an upstream target of berberine to prevent lung fibrosis during SARS-CoV and SARS-CoV-2 infection. Cyclooxygenases (COXs/PTGSs) play a significant role in many different viral infections with respect to replication and pathogenesis,[165] such as herpesviruses,[166] bovine leukemia virus,[167] and rotavirus.[168] Moreover, structural proteins from the SARS-CoV were shown to induce the expression of COX-2 in vitro[169] and elevated levels of PGE2 were found in the blood of SARS-CoV-infected individuals[170] caused by increased COX-2 expression, suggesting a role for COXs and PGs in CoV pathogenesis. Both in vitro and in vivo studies have reported that berberine decreased the expression of both TNF-α and COX-2 in a hepatotoxicity rat model induced by cyclophosphamide.[171] The inhibition of COX-2 expression[172] by berberine further increases the possibility for berberine to become a promising candidate in the prevention and treatment of COVID-19 and SARS.

3.6 | Molecular docking analysis predicts the binding modes between berberine and its crucial targets

Berberine targets can be categorized into three categories: host immune-related proteins, host receptors, and virus proteins. During SARS-CoV2 infection, the immune system is activated by stimulating lymphocytes to release cytokines including IL-6, IL-8, IL-7, IL-2, IL-1, IFN-γ, and TNF-α, which may cause hyperinflammation.[122] In addition, the cytokine

storm has been considered to be one of the major causes of acute respiratory distress syndrome (ARDS) and multiple organ failure in COVID-19 and SARS.[144] Regulating immune-related proteins is therefore likely to be beneficial for the treatment of COVID-19 and SARS. The top 10 vital proteins in the C-T-P-D network were chosen as host immune-related proteins for molecular docking analysis, including NFκB1, CHUK, MAPK3, MAPK1, NFκB1A, CASP3, IL6, MAPK8, BAX, and TNF, all of which have been shown to be strongly associated with the pathogenesis of COVID-19 and SARS (and most of which are equally prominent in PPI analysis). Blocking the viral entrance process itself is another strategy to inhibit viral infection and ACE2 has been proven to be a cellular entry receptor of COVID-19 and SARS.[173] In addition, TMPRSS2 helps the CoV spike (S) glycoprotein, a key target for the development of vaccines, therapies, and diagnostics, prime and fuse with the cellular membrane, which is crucial for SARS-CoV and SARS-CoV-2 infection and spread throughout host cells.[174] Thus, ACE2[175] and TMPRSS2 were chosen as host receptor, which are vital host targets during the viral infection process. In addition, the S protein helps SARS-CoV and SARS-CoV-2 gain entry into host cells by fusing the viral membrane with the host cell membrane.[176,177] RNA-dependent RNA polymerase (RdRp), a key part of the CoV replication machinery, is involved in processing protein production during infection.[90] RdRp has been considered one of the main drug targets against SARS-CoV and SARS-CoV-2. Moreover, PLpro and 3CLpro cysteine proteases can process polyproteins of virus, which are essential for maturation and infectivity of SARS-CoV and SARS-CoV-2.[178] Thus, spike (S), RdRp, PLpro, and 3CLpro from SARS-CoV-2[179] have been selected as the viral targets for the molecular docking to predict the binding modes of the viral proteins with berberine.

We assume that berberine regulates these targets by suppressing their gene expression or blocking binding sites at the protein level directly. Thus, molecular docking was used to calculate the binding energy and evaluate binding favorability (Table 1). The docking results show that berberine would be a promising inhibitor for each of the selected targets with moderate to strong binding affinities (−6.4--9.8 Kcal/mol), which is supported by data in the literature indicating that free binding energy greater than −5.5 kcal/mol is an indication that the compound is inactive.[180] For host immune-related proteins, the best results were obtained for the MAPK3-berberine and MAPK8-berberine complexes, with free binding energies of −8.9 kcal/mol and −8.6 kcal/mol, respectively. TNF, MAPK1, and BAX showed a moderate binding affinity with berberine, with binding energies of −8.2 kcal/mol, −8.2 kcal/mol, and −8.1 kcal/mol, respectively. The NFκB1-berberine and CHUK-berberine complexes displayed modest binding affinities of −7.3 kcal/mol in each case. By contrast, berberine exhibited weaker binding to NFκB1A, CASP3, and IL6. For host infection-related proteins, berberine showed the highest potential to inhibit the ACE2 receptor with a binding energy of −9.8 kcal/mol. Interestingly, berberine bound to the contact interface of the Spike-ACE2 complex, indicating that berberine may be an inhibitor of ACE2 enzyme activities rather than an inhibitor of ACE2-driven viral infections.[93] The binding energy of TMPRSS2-berberine was −6.7 kcal/mol, which was weaker compared with ACE2-berberine, but still notable. Berberine may also be a potential inhibitor of 3CLpro, RdRp, PLpro, and Spike, based on the modest binding energy of those complexes. It is worth mentioning that berberine has been evaluated as a potential inhibitor of 3CLpro with the lowest binding energy compared to other natural products.[181]

TABLE 1 Molecular docking results between relevant proteins with berberine

Protein type	Gene name	Affinity (Kcal/mol)
Immune-related proteins	**MAPK3**	−8.9
	MAPK8	−8.6
	TNF	−8.2
	MAPK1	−8.2
	BAX	−8.1
	NFκB1	−7.3
	CHUK	−7.3
	IL6	−6.9
	NFκB1A	−6.4
	CASP3	−6.4
Host receptor	**ACE2**	−9.8
	TMPRSS2	−6.7
Viral proteins	**3CLpro**	−6.7
	RdRp	−6.6
	PLpro	−6.6
	Spike	−6.5

The optimal binding modes of each studied berberine complex (bold shown in Table 1, ie, MAPK3-berberine, TNF-berberine, BAX-berberine, NFκB1-berberine, CHUK-berberine, ACE2-berberine, TMPRSS2-berberine, and 3CLpro-berberine) are demonstrated in Figure 6 MAPKs showed a better binding affinity with berberine in host immune-related proteins. Considering the similar structures of MAPKs, only complex MAPK3-berberine was selected and illustrated in Figure 6A, where berberine fitted well into the binding cavity of MAPK3 as a result of hydrophobic interactions with TYR53, VAL56, and LEU173. For TNF-berberine (Figure 6B), a hydrogen bond between berberine and TYR151, along with π-π stacking between berberine and TYR59, significantly contributed to the stability of the

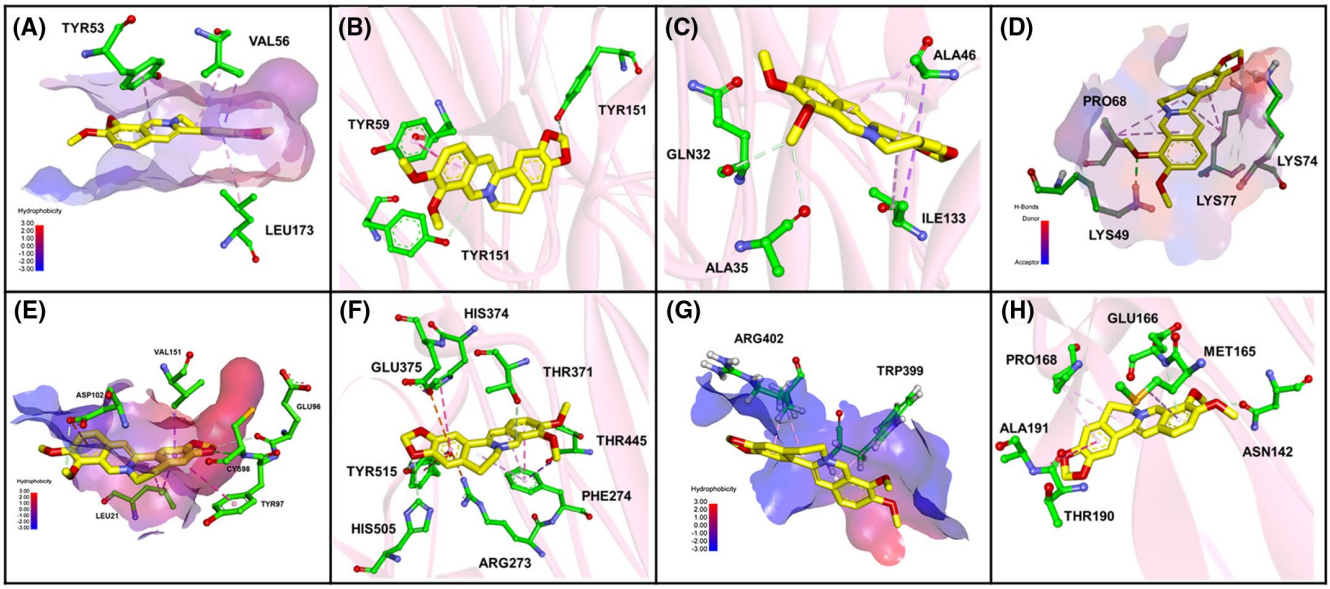

FIGURE 6 Binding explorations of complex MAPK3-berberine, TNF-Berberine, BAX-berberine, BAX-berberine, NFκB1-berberine, CHUK-berberine, ACE2-berberine, TMPRSS2 -berberine, and 3CLpro-berberine. Predicted lowest-energy binding mode of berberine with the following proteins: A, MAPK3; B, TNF; C, BAX; D, NFκB1; E, CHUK; F, ACE2; G,TMPRSS2; H, 3CLpro. For berberine, the C, O, and N are highlighted in yellow, red, and blue, respectively. For residues of proteins, the green, red, and blue stand for C, O, and N, respectively. The green, purple, and orange lines stand for hydrogen binding, hydrophobic interaction, and anion-π interaction between berberine and residues, respectively

complex. For complex BAX-berberine (Figure 6C), the hydrogen bonds between berberine with GLN32 and ALA35 stabilized the left structure of berberine, and hydrophobic bonds between berberine with ALA46 and ILE133 further stabilized the right structure of berberine.

For NFκB1-berberine (Figure 6D), hydrogen bonds with LYS49, LYS74, and LYS77 facilitated the interaction between the small molecule and its target. With respect to CHUK (Figure 6E), berberine could be successfully docked to the target, with hydrogen bonds between the berberine and CYS98, GLU96, and ASP102 further increasing the stability of the complex. For ACE2-berberine, hydrogen bonds (TYR515, HIS505, THR371, and THR445), π-π stacking (HIS374, TYR515, and PHE274), and anion-π interactions (ARG273 and GLU375) formed between protein residues and berberine contributed to stability of the complex and were clearly illustrated in Figure 6F. For TMPRSS2-berberine (Figure 6G), the berberine was attached to the hydrophobic surface of the protein. For the 3CL pro-berberine complex, key amino acid residues (ASN142, MET165, GLU166, PRO168, ALA191, and THR190) bound the berberine molecule tightly through hydrogen bonds and hydrophobic interactions (Figure 6H). More detailed two-dimensional interactions were illustrated in Figure S2.

To explain the binding significance of the berberine structure, protein ACE2 was selected to conduct molecular docking with two derivatives of berberine. Analog1 (5,6-dihydroisoquinolino[3,2-a]isoquinolin-7-ium) only retains the main skeleton without the methoxy substituent (Figure S3), while analog2 (7,8-dimethoxy-1,2,3,4-tetrahydropyrido[1,2-b]isoquinolin-5-ium) modifies the polycyclic isoquinoline-based skeleton by removing one conjugated benzene ring. The docking score of ACE2-analog1 and ACE2-analog2 are −9.3 kcal/mol and −7.4 kcal/mol, respectively. Compared to berberine, analog1 still displays most anion-π stacking and π-π stacking interactions with key protein residues, but loses the hydrogen bonds between the methoxy groups and the protein. While the binding affinity of ACE2-analog1 decreases slightly compared to ACE2-berberine, the binding affinity of ACE2-analog2 declines significantly. This implies that the anion-π stacking and π-π stacking interactions formed by the conjugated skeleton of berberine and residues from ACE2 are critical features in a tightly binding complex between the small molecule and ACE2.

3.7 | Berberine/NIT-X inhibits SARS-Cov-2 replication, ACE2, and TMPSS2 gene expression in SARS CoV-2-infected human lung epithelial cell line

To provide experimental support for the hypothesis that berberine/NIT-X could be an effective inhibitor SARS-Cov-2, the replication level of SARS-CoV-2 in infected calu-2 cells

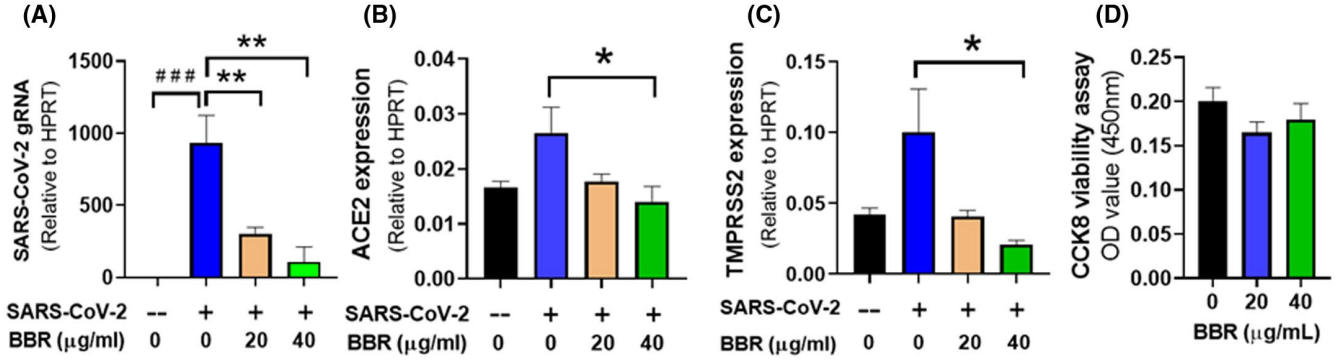

FIGURE 7 Effect of BBR (berberine/NIT-X) on ACE2, TMPRSS2 expression in infected Calu-3 cells. Calu-3 cells were seeded in six well plates with 5×10^5 cells per well. After 24 hours, cells were incubated in media containing 20 μg/mL, 40 μg/mL BBR/NIT-X or DMSO (v/v 1:1000). After 3 days incubation, cells were infected with SARS-CoV-2 (MOI = 0.005) or mock (-) for 1hr and grown in indicated media for 24 hours. One day post infection, total RNA were for cDNA synthesis. Real Time-PCR was performed with the primer for SARS-CoV-2 (A) gRNA of SARS-CoV2, (B) ACE2, and (C) TMPRSS2. Data were normalized to HPRT and presented as 2-ΔCT. Data represent two sets of qPCR with six readouts. D, Cytotoxicity was performed using commercial CCK8 toxicity kit, $^*P < .05$ versus infected, but not treated. $^{\#}P < .05$ versus uninfected/untreated

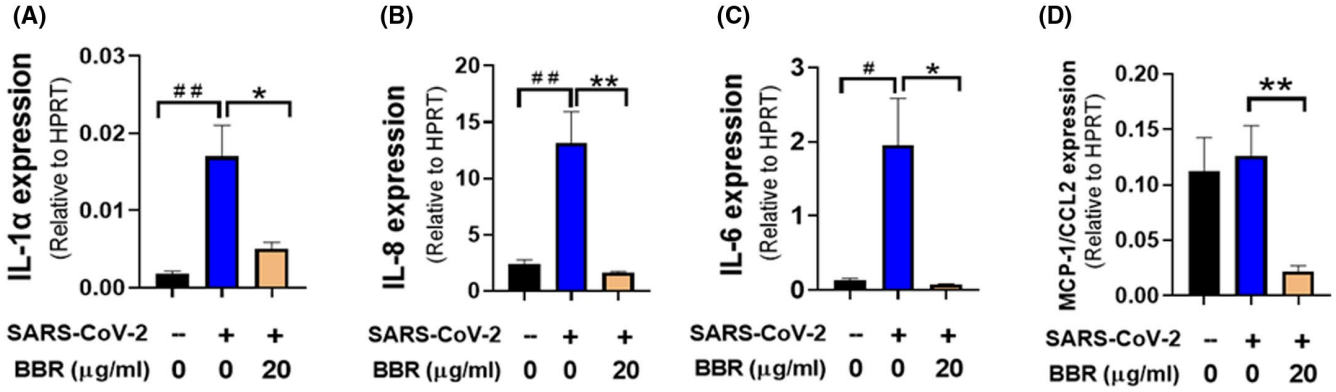

FIGURE 8 Effect of BBR (berberine/NIT-X) cytokine expression in infected Calu-3 cells. Calu-3 cells were cultured, treated, infected and qPCR were performed. $^*P < .05$,$^{**}P < .01$ versus infected, but not treated. Data represent two sets of qPCR with six readouts. $^{\#}P < .05$ versus uninfected/untreated

was evaluated by detecting SARS-CoV-2 N protein gene expression using qPCR. We observed that 1 day post infection, there was a marked increase in SARS-CoV-2 N protein gene expression in SARS-CoV-2 infected cells compared with mock infected Calu-3 cells (Figure 7A, $P < .01$). Berberine/NIT-X treatment at both 20 μg/mL and 40 μg/mL significantly inhibited the viral levels (Figure 7A, $P < .01$ vs. untreated). ACE2 and TMPSS2 levels were further analyzed and an increasing trend was observed for both genes when compared with noninfected cells. We next determined whether berberine/NIT-X inhibits viral infection (entry) by suppression of ACE2 and TMPSS2 expression, with data showing that berberine/NIT-X inhibited the ACE2 and TMPSS2 levels at both 20 μg/mL and 40 μg/mL (Figure 7B,C, $P < .05$ vs. un-treated). No cell toxicity was observed at these two concentrations (Figure 7D). The data further validate our hypothesis that berberine inhibits viral infection by regulating host receptor ACE2 and TMPSS2.

3.8 | Berberine/NIT-X inhibits inflammatory cytokine and chemokine gene expression by SARS-CoV-2 infected human lung epithelial cells line

We also investigated the effect of berberine/NIT-X on the expression of different cytokines and chemokine in infected Calu3 cells; specific lung gene expression differentiation of SARS and COVID-19 were illustrated in (Figure S4). There are 12 and 15 pharmacological targets for SARS and COVID-19, respectively, all of which are potential berberine targets. Among them, eight shared targets (IL1α, IL6, IL8, CCL2, PTGS2, NLRP3, VEGFA, and ERBB2) were displayed in the overlap area. IL-1α, IL-8, IL-6, and CCL2 are shared genes that were selected as targets of berberine for further validation. The data showed that the IL-1α, IL-8, and IL-6 gene expression were significantly increased in virus infected, nontreated cells. After the treatment of

berberine/NIT-X at 20 μg/mL, the expression of IL-1α, IL-8, and IL-6 were all significantly decreased (Figure 8A-C, $*P < .05$; $**P < .01$). No significant difference was observed in CCL-2 levels in untreated infected cells versus untreated noninfected cells; however, under the treatment of berberine/NIT-X, CCL-2 expression was significantly reduced (Figure 8D, $**P < .01$). These data demonstrate that berberine/NIT-X effectively suppresses the expression of pro-inflammatory cytokines and chemokines including IL-1α, IL-8, IL-6, and CCL2, which reduce the risk of cytokine storm and pneumonia in COVID-19. The data further support our hypothesis that berberine can inhibit various pro-inflammatory cytokines and protect against tissue damage during viral infection.

4 | CONCLUSION

We have identified potential therapeutic targets of berberine against both SARS and COVID-19 using computational modeling. The most prominent targets for berberine relevant to host immune response include NF-κB and MAPKs, which are important proteins regulating the cytokine storm, and CASPs and BAX, which are relevant targets in preventing tissue damage via suppressing cell death signaling pathways. Besides balancing host immune responses, our molecular docking analysis identifies berberine as a potential antagonist of host receptor for viral entry, such as ACE2 and TMPSS2, and may inhibit virus proteins. Furthermore, as the first step to validate our computational modeling results, we for the first time demonstrate that berberine significantly reduced viral replication, suppressed viral entry host receptor ACE2 and TMPSS2, and decreased inflammatory markers including IL-6, IL-8, IL-1α, and CCL2 in SARS-CoV-2 infected lung epithelial cells. Given that berberine/NIT-X exhibits high oral bioavailability and has previously been shown to have in vivo immunomodulatory effects and suppression of hypermast cells activation, berberine/NIT-X has the potential to become a promising, orally active therapeutic against COVID-19 and SARS. However, direct evidence of berberine antagonist to ACE2, TMPRSS2 protein, and binding activities with these receptors and other targets have not been elucidated in this study and will be further investigated in our future research.

Berberine/NIT-X may exhibit a number of possible clinical prospective applications as follows: (1) Given that berberine improves the Th1 immune response at the early stage of infection, then, inhibits inflammatory responses triggered by viruses at the late stage, it is possible that berberine can be both a preventive and treatment option for individuals at a higher risk of viral infection such as immune-compromised patients. (2) Our computational modeling showed that berberine targets a cancer pathway, an AGE-RAGE signaling pathway in diabetic complications, and a number of other viral infection-related immune response pathways. Therefore, berberine/NIT-X may have be potentially useful for patients with preexisting condition such as cancer, diabetes, and patients with other viral infection including influenza, EBV, CMV, HBV, etc. (3) Clinically, a high dosage of berberine may cause gastrointestinal side-effects. berberine/NIT-X boosts oral bioavailability and reduces the dosage of oral administration by 6 times, and thereby may evade the side-effects caused by high dosage. (4) Berberine might regulate host immune responses, inhibit host viral receptors, and block virus proteins to prevent and treat SARS and COVID-19. Therefore, the co-treatment with immune supplements such as ascorbic acid, antiviral drugs such as remdesivir, or antibodies from convalescent plasma might be interesting avenues to enhance the effects of berberine. Recently, strong synergistic in vitro antiviral activities between remdesivir and berberine have been reported,[182] which further supports our hypothesis.

Taken together, our current study may lead to berberine/NIT-X as an orally active therapeutic candidate in the prevention and treatment of COVID-19, SARS, and other viral infections. In vitro and in vivo studies are currently in progress to investigate, confirm, and expand the knowledge into the molecular alterations induced by berberine in preventing and treating COVID-19 and SARS.

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CONFLICT OF INTEREST

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US14/857772, PCT/US14/68396, PCT/US2014/012306, PCT/US2005/008417, and (PCT/US2017/056822 (Pending), is a member of Herbs Springs, LLC, General Nutraceutical Technology LLC, and Health Freedom LLC. NY shares US patent PCT/US14/68396 and is a member of Health Freedom LLC. KS share patent (PCT/US2017/056822 (Pending), Salary support by General Nutraceutical Technology LLC.

AUTHOR CONTRIBUTIONS

Z.-Z. Wang and X.-M. Li designed this study. Z.-Z. Wang, K. Li, N. Yang, and K. Srivastava performed the data collection, analysis, target validation, and manuscript preparation. A.R. Maskey helped prepare manuscript. A.A. Toutov, W. Huang, M. Miao, N. Yang, K. Srivastava, R. Tiwari, J. Geleibter, and X.-M. Li contributed to the manuscript preparation/revision and provided guidance. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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