Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Antiviral fungal metabolites and some insights into their contribution to the current COVID-19 pandemic

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak, which started in late 2019, drove the scientific community to conduct innovative research to contain the spread of the pandemic and to care for those already affected. Since then, the search for new drugs that are effective against the virus has been strengthened. Featuring a relatively low cost of production under well-defined methods of cultivation, fungi have been providing a diversity of antiviral metabolites with unprecedented chemical structures. In this review, we present viral RNA infections highlighting SARS-CoV-2 morphogenesis and the infectious cycle, the targets of known antiviral drugs, and current developments in this area such as drug repurposing. We also explored the metabolic adaptability of fungi during fermentation to produce metabolites active against RNA viruses, along with their chemical structures, and mechanisms of action. Finally, the state of the art of research on SARS-CoV-2 inhibitors of fungal origin is reported, highlighting the metabolites selected by docking studies.

1. Introduction

The world is facing the rapid spread of a novel virus, designated as SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), the causative agent of coronavirus disease 2019 (COVID-19). The outbreak, which started in December 2019 in Wuhan, China, quickly emerged as a global threat, urging coordinated efforts of authorities, physicians, and scientists to better understand the spread of the virus, and to find potential therapeutics and prophylactics to minimize the socioeconomic and public health effects of such diseases. SARS-CoV-2 was included among the priority viruses in the 2020 WHO Research & Development Blueprint.[1] In the fight against viruses and diseases, there is a huge demand for strategies to increase people's adherence to reliable models that can decrease virus transmission. By July 20, 2021, SARS-CoV-2 has infected over 190.6 million people worldwide, with over 4 million deaths, and the number is still growing rapidly.[2]

Since complete virus eradication is not possible, the search for vaccines directed to prophylaxis strategies and antiviral drugs to treat affected patients is a worldwide priority. Effective drugs to combat SARS-CoV-2 and cure COVID-19 are still being developed, and vaccines are the first choice to contain the spread of the infection. In July 2021, there were 105 COVID-19 vaccine candidates in clinical trials or already being used (see Table 1 for clinical trials).[3,4] However, only 13 vaccines received rapid temporary regulatory approval to address significant public health issues such as pandemic. The vaccine produced by BioNTech in cooperation with Pfizer (BNT162b2/COMIRNATY – INN Tozinameran) was approved in December 2020 and the others by the beginning of 2021.[3–6]

Although vaccination is in progress in almost all countries, with more than 2.9 million vaccine doses administered from the end of 2020 to July 2021[2], mass immunization will not be achieved so quickly to effectively control the pandemic, since several countries cannot afford the costs and logistics for vaccine distribution, stocking, and preservation. In addition to the inherent challenges of this process, the world faces an additional threat due to new SARS-CoV-2 genetic variants with higher virulence, which in certain cases may present the ability to escape

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Table 1
COVID-19 vaccines registered on OMS and/or currently used worldwide.

Published name [Clinical trials registered]	Developer (Country of origin)	Countries of authorization/ approval
Type: Inactivated SARS–CoV-2		
CoronaVac (SARS-CoV-2 vaccine) [NCT04352608, NCT04383574, NCT04800133, NCT04456595, NCT04582344, NCT04508075, NCT04756830]	Sinovac Research and Development Co. Ltd. (China)	Albania, Armenia, Azerbaijan, Bangladesh, Bolivia, Bosnia and Herzegovina, Botswana, Brazil, Cambodia, China, Chile, Colombia, Dominican Republic, Ecuador, Egypt, Georgia, Hong Kong, Indonesia, Kazakhstan, Laos, Libya, Malaysia, Mexico, Moldova, Nepal, Pakistan, Panama, Paraguay, Philippines, Singapore, South Africa, Thailand, Timor-Leste, Togo, Tunisia, Turkey, Turkmenistan, Ukraine, Uruguay, Zimbabwe, WHO
BBIBP-CorV [ChiCTR2000032459, NCT04560881, NCT04863638, ChiCTR2000034780, ChiCTR2100041704]	Sinopharm + China National Biotec Group Co + Beijing Institute of Biological Products (China)	Afghanistan, Algeria, Angola, Argentina, Bahrain, Bangladesh, Barbados, Belarus, Bolivia, Bosnia and Herzegovina, Brazil, Brunei, Cambodia, Cameroon, Chad, China, Congo, Dominican Republic, Egypt, Ethiopia, Equatorial Guinea, Gabon, Georgia, Guyana, Hungary, Indonesia, Iraq, Jordan, Kazakhstan, Kyrgyzstan, Laos, Macau, Maldives, Mauritania, Moldova, Mongolia, Montenegro, Morocco, Mozambique, Myanmar, Namibia, Nepal, Niger, North Macedonia, Pakistan, Papua New Guinea, Peru, Philippines, Senegal, Serbia, Seychelles, Sierra Leone, Solomon Islands, Somalia, Sri Lanka, Sudan, Thailand, Turkmenistan, UAE, Vanuatu, Venezuela, Vietnam, WHO, Zambia, Zimbabwe
Covaxin (BBV152) [NCT04471519, NCT04641481, CTRI/2020/11/028976, NCT04918797]	Bharat Biotech + India's National Institute of Virology ICMR + Ocugen + ViroVax (India)	Botswana, Estonia, Guatemala, Guyana, India, Iran, Mauritius, Mexico, Myanmar, Nepal, Paraguay, Philippines, Venezuela, Zimbabwe
WIBP-CorV [ChiCTR2000031809, NCT04885764, ChiCTR2000034780]	Sinopharm + China National Biotec Group Co + Wuhan Institute of Biological Products (China)	China
CoviVac	Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products (Russia)	Russia
QazVac (QazCovid-in) [NCT04530357, NCT04691908]	Research Institute for Biological Safety Problems (Kazakhstan)	Kazakhstan
Unnamed vaccine candidate [NCT04758273, NCT04756323, NCT04852705]	Minhai Biotechnology Co. + Shenzhen Kangtai Biological Products Co. Ltd. (China)	China
Unnamed vaccine candidate [NCT04412538, NCT04470609, NCT04659239]	Chinese Academy of Medical Sciences (China)	China
COVIran Barekat [IRCT20201202049567N1, IRCT20201202049567N3]	Shifa Pharmed Industrial Group (Iran)	Iran
Type: SARS–CoV-2 protein unity		
Abdala (CIGB 66) [IG/CIGB-66I/CVD19/2002, IG/CIGB–66I/CVD19/2103]	Center for Genetic Engineering and Biotechnology (Cuba)	Cuba, Venezuela
EpiVacCorona [NCT04527575, NCT04780035]	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology (Russia)	Belarus, Russia Turkmenistan
Type: Recombinant protein subunit		
ZF2001 [NCT04445194, NCT04466085, NCT04550351, NCT04646590, NCT04833101]	Anhui Zhifei Longcom Biopharmaceutical (Uzbekistan) + Institute of Microbiology, Chinese Academy of Sciences (China)	China, Uzbekistan
Type: Conjugated protein subunit		
Soberana 02 [RPCEC00000340, RPCEC00000347]	BioCubaFarma + Finlay Institute of Vaccines (Cuba)	Cuba, Iran
Type: Non-replicating recombinant adenovirus vector		
COVID-19 Vaccine AstraZeneca (Covishield, Vaxzevria AZD1222, ChAdOx1-S) [PACTR202005681895696, PACTR202006922165132, NCT04686773, NCT04324606, NCT04400838, ISRCTN89951424, NCT04516746, NCT04760132, NCT04794946]	AstraZeneca + University of Oxford (UK)	Afghanistan, Albania, Algeria, Andorra, Angola, Argentina, Armenia, Australia, Bahamas, Bahrain, Bangladesh, Barbados, Bhutan, Bolivia, Botswana, Brazil, Brunei,

(*continued on next page*)

Table 1 (*continued*)

Published name [Clinical trials registered]	Developer (Country of origin)	Countries of authorization/ approval
		Cabo Verde, Cambodia, Canada, Cambodia, Caribbean, Chile, Colombia, Congo, Costa Rica, Djibouti, Dominican Republic, Ecuador, El Salvador, Egypt, Estonia, Eswatani, Ethiopia, European Union, Faroe Islands, Fiji, Gambia, Georgia, Ghana, Greenland, Guatemala, Guinea-Bissau, Guyana, Honduras, Hungary (SII), Iceland, India, Indonesia, Iran, Iraq, Ivory Coast, Japan, Jordan, Kenya, Kosovo, Kuwait, Lebanon, Lesotho, Liberia, Libya, Malawi, Malaysia, Maldives, Mali, Mauritius, Mexico, Moldova, Mongolia, Morocco, Myanmar, Namibia, Nepal, Nicaragua, Nigeria, North Macedonia, Norway, Oman, Pakistan, Palestine, Panama, Papua New Guinea, Peru, Philippines, Rwanda, Saint Vincent and the Grenadines, Samoa, Serbia, Seychelles, Sierra Leone, Somalia, South Korea, South Sudan, Sri Lanka, Sudan, Suriname, Taiwan, Tajikistan, Thailand, Timor Leste, Tonga, Togo, Tuvalu, Uganda, Ukraine, UK, Uzbekistan, Vietnam, WHO (Oxford; SII/SK), Yemen, Zambia
Type: Non-replicating recombinant adenovirus vector (rAd26 and rAd5)		
Sputnik V (Gam-COVID-Vac) [NCT04436471, NCT04437875, NCT04587219, NCT04564716, NCT04530396]	Gamaleya Research Institute + Health Ministry of the Russian Federation + Acellena Contract Drug Research and Development (Russia)	Albania, Algeria, Angola, Antigua and Barbuda, Argentina, Armenia, Azerbaijan, Bahrain, Bangladesh, Belarus, Bolivia, Brazil, Congo, Djibouti, Ecuador, Egypt, Gabon, Ghana, Guatemala, Guinea, Guyana, Honduras, Hungary, India, Iran, Iraq, Jordan, Kazakhstan, Kenya, Kyrgyzstan, Laos, Lebanon, Maldives, Mali, Mexico, Moldova, Mongolia, Montenegro, Morocco, Myanmar, Namibia, Nicaragua, North Macedonia, Pakistan, Palestine, Panama, Paraguay, Republika Srpska, Russia (Sputnik Light), Saint Vincent and the Grenadines, San Marino, Serbia, Slovakia, Sri Lanka, Syria, Tunisia, Turkey, Turkmenistan, United Arab Emirates, Uzbekistan, Venezuela, Zimbabwe
Type: Non-replicating recombinant adenovirus vector (rAd26)		
Sputnik Light [(NCT04713488, NCT04741061]	Gamaleya Research Institute + Health Ministry of the Russian Federation + Acellena Contract Drug Research and Development (Russia)	Angola, Bahrain, Congo, Kyrgyzstan, Mauritius, Mongolia, Nicaragua, Palestine, Russia, Venezuela
Type: Non-replicating recombinant adenovirus vector (Ad5)		
Convidicea (PakVac, Ad5-nCoV) [ChiCTR2000030906, ChiCTR2000031781, NCT04526990, NCT04892459, NCT04916886]	CanSino Biologics (China) + Beijong Institute of Biotechnology	Argentina, Chile, China, Ecuador, Hungary, Malaysia, Mexico, Moldova, Pakistan
Type: Non-replicating recombinant viral vector		
COVID-19 Vaccine Janssen (JNJ-78436735; Ad26.COV2.S) [NCT04509947, NCT04535453, NCT04436276, EUCTR2020-002584–63-DE, NCT04614948, NCT04505722, EUCTR2021-002327–38-NL, NCT04817657]	Janssen Bioech (Johnson & Jonhson) (The Netherlands, US)	Andorra, Australia, Bahrain, Bangladesh, Botswana, Brazil, Canada, Chile, Colombia, Denmark, EU, Faroe Islands, Greenland, Iceland, India, Kuwait, Liechtenstein, Malaysia, Maldives, Mexico, Moldova, New Zealand, Nigeria, Norway, Philippines, Saint Vincent and the

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Table 1 (*continued*)

Published name [Clinical trials registered]	Developer (Country of origin)	Countries of authorization/ approval
		Grenadines, South Africa, South Korea, Switzerland, Thailand, Tunisia, United Kingdom, US, WHO, Zambia
Type: mRNA based **Comirnaty** (BNT162b2) [NCT04523571,2020–001038-36, NCT04588480, NCT04649021, NCT04754594, NCT04816643, NCT04368728, NCT04761822, NCT04760132, NCT04844489]	Pfizer/BioNTech + Fosun Pharma (multinacional)	Albania, Andorra, Argentina, Aruba, Australia, Bahrain, Bangladesh, Bosnia and Herzegovina, Brazil, Brunei, Canada, Caribbean, Chile, Colombia, Costa Rica, Ecuador, European Union, Faroe Islands, Greenland, Hong Kong, Iceland, India, Iraq, Israel, Japan, Jordan, Kuwait, Lebanon, Liechtenstein, Macao, Malaysia, Maldives, Mexico, Moldova, Monaco, Mongolia, New Zealand, North Macedonia, Norway, Oman, Palestine, Pakistan, Panama, Peru, Philippines, Qatar, Rwanda, Saint Vincent and the Grenadines, Saudi Arabia, Serbia, Singapore, South Africa, South Korea, Sri Lanka, Suriname, Switzerland, Thailand, Tunisia, Turkey, Ukraine, UAE, UK, US (16 and older), Vatican City, Vietnam, WHO
Moderna COVID-19 Vaccine (mRNA-1273.351, Spikevax, TAK-919, Elasomeran) [NCT04785144, NCT04847050, NCT04283461, NCT04405076, NCT04470427, NCT04649151, NCT04761822, EUCTR2021-000930–32]	Moderna + Biomedical Advanced Research and Development Authority (BARDA) + National Institute of Allergy and Infectious Diseases (NIAID) (USA)	Andorra, Australia, Bangladesh, Brazil, Botswana, Canada, Colombia, European Union, Faroe Islands, Greenland, Guatemala, Honduras, Iceland, India, Indonesia, Israel, Japan, Liechtenstein, Maldives, Moldova, Mongolia, Norway, Palestine, Philippines, Qatar, Saint Vincent and the Grenadines, Singapore, South Korea, Switzerland, Taiwan, Thailand, United Kingdom, UAE, United States, Vietnam, WHO

Data updated on July 2021 from WHO[3] and Craven[4]

from the vaccine-driven immune response.[7,8] Therefore, current measures to decrease pandemic spread, avoid the insurgence of new variants, and reduce the number of deaths still include social distancing and other behavioral changes, as well as the search for effective drugs for the treatment of infected individuals to ensure patient recovery.

In addition, other viral threats for humankind that afflict restricted groups of individuals or are endemic in specific world regions have been re-emerging, which is also very concerning because of high lethality, severe side effects, high cost of medicines, and absence of effective vaccines. A relevant issue in the re-emergence of viral threats is the intense aerial transportation, migration, and inadequate hygienic-sanitary conditions that contribute to virus dissemination. Hepatitis virus (HV) types A (HAV), B (HBV), C (HCV), D, and E are good examples of major public health concerns, and immediate actions are necessary to achieve the World Health Organization's goal of eliminating the disease as a public health threat by 2030.[9] Acquired Immune Deficiency Syndrome (AIDS) epidemic caused by Human Immunodeficiency Virus (HIV) has become a major concern in the last 40 years, but, in this case, antiretroviral therapy helped to decrease the number of deaths related to HIV.[10] Outbreaks of other emerging or reemerging viruses such as Dengue virus (DENV), Severe Acute Respiratory Syndrome (SARS) virus, Middle East Respiratory Syndrome coronavirus (MERS-CoV), and Influenza A virus (IAV) have been reported.[13–18] Fig. 1 presents data on some viruses of worldwide concern.

The reemergence of viral diseases is associated with virus genetic flexibility, especially in fast-mutating RNA viruses, giving them the ability to adapt to new hosts and environments, leading to different epidemiological behaviors at local and global levels.[11] These events allied to their anthropogenic causes,[12] together with the underlying processes of virus mutation[11] and adaptation offer good epidemiological models that could help to predict future pandemic reemergence.

In this review, we discuss some antiviral drugs currently available, the activity and structural features of selected fungal metabolites effective against RNA viruses, and the structures of some interesting antiviral fungal metabolites. Some parallels with demands related to COVID-19 medications are also shown. We hope to shed some light on the huge number of fungal metabolites active against emerging and reemerging viruses, especially to pave the way to conquer COVID-19.

2. An overview of fungal metabolites as drug leads

Among the initiatives to control the spread of viral diseases and the severity of their effects, the development of new antivirals is critical. In this scenario, drug repurposing has been a useful strategy, since toxicity can be anticipated, and industrial production has already been established.[16] This is the case of two fungal metabolites, cyclosporine A and mycophenolic acid, both previously approved by the US Food and Drug Administration (FDA) as immunosuppressant agents, which became drug candidates against HIV-1, and Vesicular Stomatitis Indiana Virus (VSV).[18] The synthesis of mycophenolic acid derivatives with different functionalities in the aromatic cycle has been reported to overcome side effects and toxicity.[19]

Fungal metabolites are also promising for the development of new antiviral drugs. The production of fungi-derived metabolites can be scaled up for industrial manufacture of drugs with relatively low

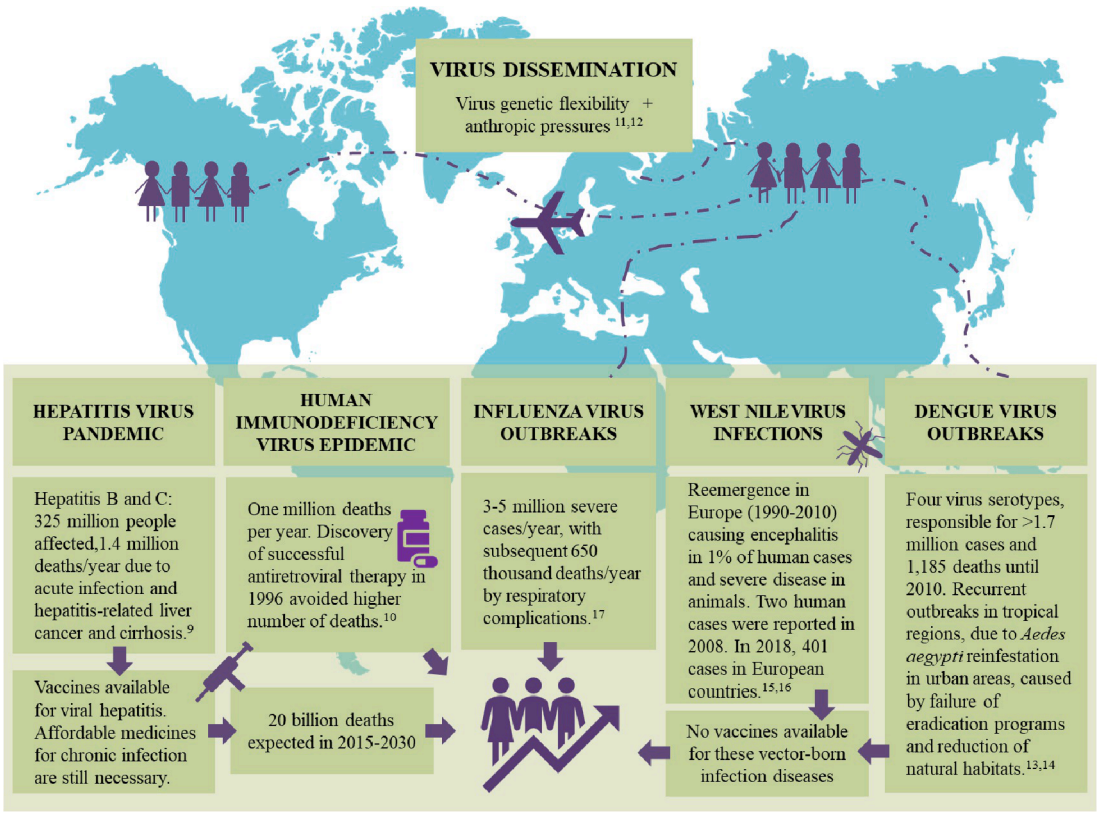

Fig. 1. Emergence and reemergence of viral diseases worldwide.

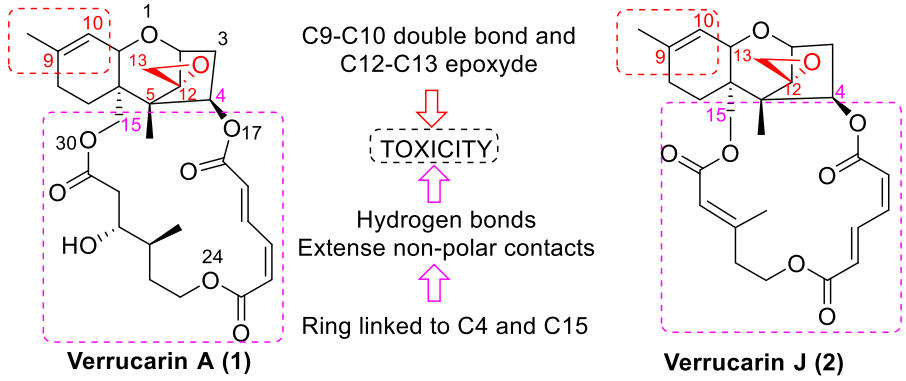

Fig. 2. Chemical structures of verrucarins A (**1**) and J (**2**), trichothecenes with antiviral potential, highlighting structural features related to toxicity.

production costs compared to the development of synthetic drugs, and some early related fungal metabolites are worth revisiting. For instance, in the 1960s, statolon, a polysaccharide produced by *Penicillium stoloniferum*, was shown to induce interferon production by murine immune cells in response to Friend Leukemia Virus infection, without strong outcomes at that time.[20] After further studies, statolon is currently listed in a patent as an active antiviral compound.[21] Another example comprises an early reported group of antiviral macrocyclic trichothecene mycotoxins, such as verrucarin A (**1**). Although trichothecenes toxicity, verrucarin J (**2**) is currently being studied as a drug against lung cancer,[22] while verrucarin A (**1**) has been reported as one of the three top drugs to bind SARS-CoV-2 proteinase in docking studies.[23] Verrucarin J and A toxicity is related to several structural factors, such as the double bond between C9 and C10, the epoxide ring between C12 and C13, the number of oxygen substituents, and macrocycle ester functions (Fig. 2).[24]

In another example, chetomin (**3**) (Fig. 3), a diketopiperazine dimer isolated from *Chaetomium cristatumis*, demonstrated potential for inhibiting VSV[25] and toxicity associated with the sulfide bridge, present in this class of molecules, were initially limiting factors for the development of chetomin-based drugs. Based on structure–activity relationship (SAR) studies, less toxic simplified related compounds have been developed with the epidithiodiketopiperazine moiety present in the natural compound chaetocin (**4**), such as PSETP-1 (**5**).[26] Meanwhile, thiodiketopiperazine compounds have been designed and elegantly synthesized using a stereoselective approach, leading to promising antitumor compounds with reduced toxicity.[27] Fig. 3 presents the chemical structures of compounds **3**, **4**, and **5**, highlighting the structural features related to antiviral activity (in red) and toxicity (in black and green).

Therefore, even fungal metabolites previously discarded from clinical trials, due to toxicity issues, may still become drug leads using techniques such as structural simplification and synthesis of derivatives, to achieve less toxic semi-synthetic analogs. These examples

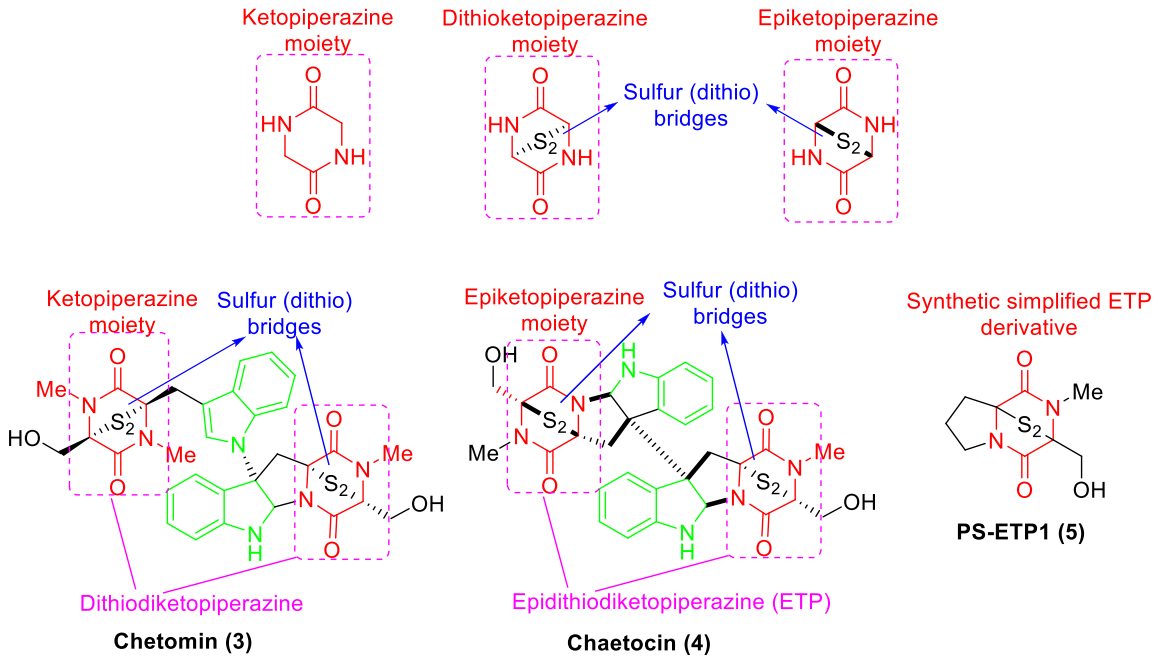

Fig. 3. Structures of diketopiperazines **3**, **4**, and **5**, with reference to their classification and features related to toxicity (sulfur bridges).

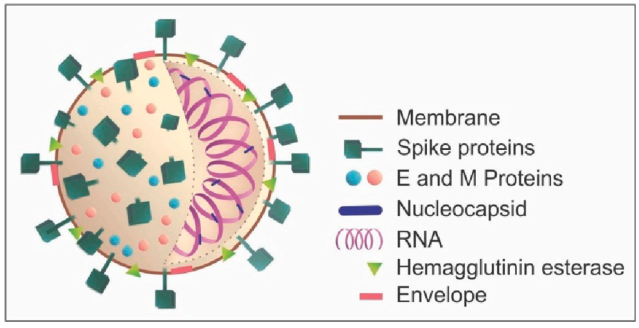

Fig. 4. Schematic representation of Coronaviridae viral particle structure. The SARS-CoV-2 surface proteins (spike, envelope (E), membrane (M), nucleocapsid, hemagglutinin), and the (+) sense single-strand helicoidal RNA are represented.

demonstrate the value of a new insight over the state-of-the-art of research on fungal metabolites with antiviral activity, directed toward the discovery of effective new prescription drugs for individuals infected with SARS-CoV-2.

3. SARS-CoV-2 infection and COVID-19

Aside from the fact that the Coronaviridae family has been known for almost five decades, specific treatments concerning drugs or vaccination are emerging for SARS-CoV-2 in 2021. Possible therapeutic targets for COVID-19 depend on the molecular mechanisms underlying viral replication, genome sequencing, and proteome identification.[28] The approach "Breaking the Cycle" (the reproductive cycle) of IAV, HIV, HV, and SARS-CoV-2, based in the diversity of viral families and their specific molecular strategies for host-cell infection and particle replication, has raised important research strategies for treating viral diseases, and it is directing COVID-19 treatment.[29]

Viruses are supramolecular complexes of nucleic acids, either DNA or RNA, encapsulated in a protein coat that may contain proteases or polymerases that are necessary for viral replication inside the cell. SARS-CoV-2 particles (Fig. 4) are protected by a lipid envelope (E), where several proteins with structural and virulence importance [membrane proteins (M), spike proteins (S), and hemagglutinin esterase] are anchored.[30] Viruses can infect organisms of a broad taxa spectrum, with different tropisms. Viral-host specificity is determined by surface proteins (e.g., spike proteins and host receptors) that trigger the adsorption (virus-host interaction) process. In general, host-cell infection occurs via cell fusion between the host-cell plasma membrane and the viral particle or via receptor-mediated endocytosis with particle internalization (endosome). Untied endosomes allow for the release of viral particles inside the cell. Retroviruses replicate through the mediation of complementary DNA (cDNA), copied from the viral RNA genome by reverse transcriptase, and integrated into the chromosomal DNA of the host cells. (−) RNA strands are synthesized by the host, using the viral DNA code integrated into the cell genome, which serve as a template for the synthesis of complementary (+) RNA strands. The latter are packaged into new virus particles. Viral morphogenesis occurs after the synthesis of proteins from the virus repertoire, using the molecular machinery of the host cells. This step involves the movement of the cytoskeleton for cell remodeling, and impairment of physiological functions of the infected cell.

Considering the extensive differences between virus species reproduction cycles (e.g., enveloped versus non-enveloped virus, DNA versus double-RNA genome), the infectious cycle of SARS-CoV-2, an enveloped virus with a positive-sense single-stranded RNA genome, is briefly presented. The SARS-CoV-2 reproduction cycle, from its early stages to virion particle release, is schematically represented in Fig. 5. Viral infection begins with a random collision between a viral particle and a potential host cell. Before entering, either via endosomal or membrane fusion, SARS-CoV-2 spike proteins must interact with angiotensin-converting enzyme 2 (ACE2) cell receptors to invade the cell.[31] S–glycoprotein-ACE2 engagement is dependent on the proteolytic activity of cathepsin L, an endosomal endopeptidase, and transmembrane protease serine 2 (TMPRSS) when cell invasion occurs via membrane fusion. S-cleavage into S1/S2 subunits is essential for host cell invasion and genome release (uncoating).[31] ACE2, which is part of the angiotensin vasoconstriction regulatory axis, is abundantly present in lung epithelial cells, and is therefore implicated in the severe pathorespiratory condition of COVID-19.[32]

Once inside the host cell, the viral genome is released. The SARS-

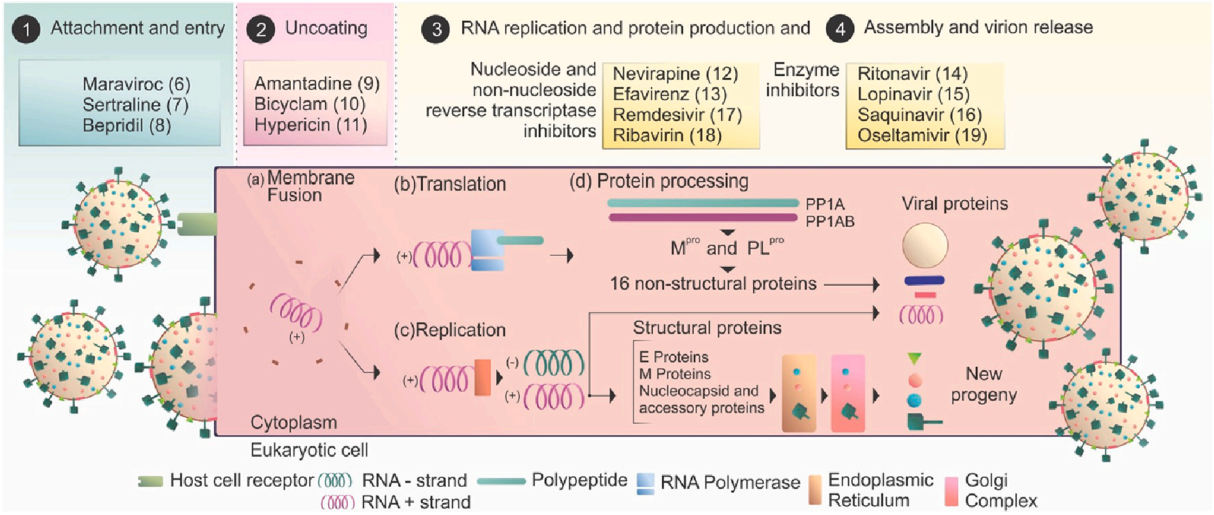

Fig. 5. SARS-CoV-2 reproduction cycle. The schematic cycle of enveloped betacoronavirus divided into four main infection phases. (1) Attachment and entry: viral recognition of the ACE2 host-cell receptor proceeds by spike protein cleavage (S1/S2) for cell invasion engagement. (2) Genome uncoating: after cell fusion or endosomal penetration, the viral genome is released in the host-cytoplasm with nucleocapsid untied. (3) RNA replication and protein production: the main phase in the viral invasion is the use of the host-cell apparatus for virion particle reproduction. The RNA genome is replicated for new particle assembly and translated for viral-protein production including autocatalytic proteases (M^{pro} and PL^{pro}), composing an arsenal of 16 non-structural proteins for replicase assemble. (4) Final process: reorganization of the envelope membrane and the newly synthesized proteins and genome for virion particle release via exocytosis. Some compounds that inhibit viral reproduction are indicated.

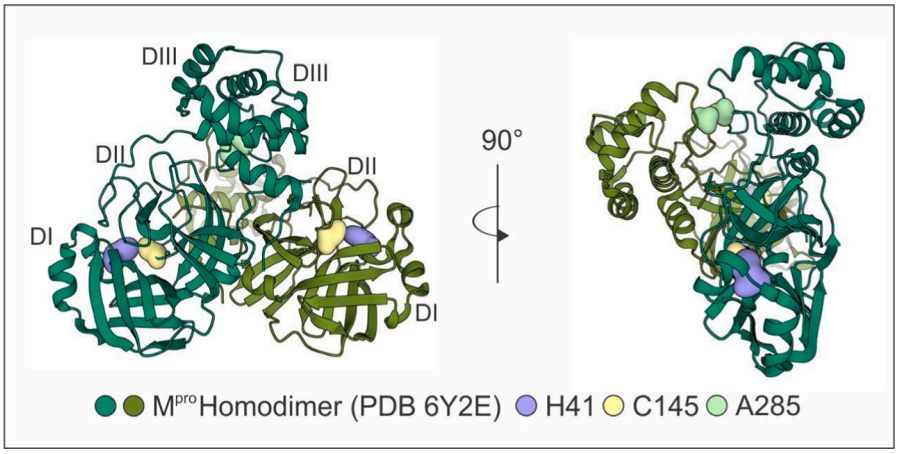

Fig. 6. SARS-CoV-2 Main Protease 3D structure. M^{pro} functional protein is organized on a homodimer. Each monomer includes three different functional domains (DI, DII, and DII). The catalytic cleft is between DI and DII with a His41 and C145 catalytic dyad. DIII is involved in homodimer stabilization. A285 residues differ from SARS-CoV M^{pro} homolog and contribute to protease activity improvement. Reference protein model PDB 6Y2E.[38.]

CoV-2 genome contains approximately 30,000 nucleotides and encodes for structural (e.g., envelope proteins) and non-structural proteins (e.g., helicases), including a self-RNA replication/transcription complex (RTC).[33] The replicase complex is encoded by a 20 kb gene in the positive-sense single-stranded RNA. In the host-cell cytoplasm, the coronavirus replicase complex gene is translated into two polyproteins, PP1A and PP1AB, which include 16 non-structural proteins. PP1A and PP1AB are edited by SARS-CoV-2 main protease (M^{pro}), also known as chymotrypsin-like protease ($3CL^{pro}$), and papain-like protease (PL^{pro}).[30] M^{pro} recognizes eleven cleavage points, while PL^{pro} has three predicted cleavage sites over the polyproteins.[34] Genome replication and viral particle assembly are highly dependent on both proteases. The newly synthesized viral protein structural apparatus develops inside the endoplasmic reticulum and the Golgi apparatus, and sends to progeny assembly, together with genome replicates, providing novel virion particles that are released via exocytosis.[35] Most of them are prompted by plasma membrane depolymerization, which can be completely ruptured or used to form a lipid envelope around the capsid. This process, even if it does not lyse the cell, makes it infeasible. Extensive changes in host cell physiology and morphology are associated with the cytopathic effects resulting from a viral infection, such as cell lysis, apoptosis initiation, host-cell protein synthesis blockade, membrane transport interruption, cytoskeleton disruption, and host-cell intensive metabolic state. Virulence determines the extension of replication and disease transmission in viruses before host cell failure.[36]

4. The SARS-CoV-2 protease – The main target for antiviral drugs

Because of the importance of viral proteases for the transcription and replication of Coronavirus, this family of proteins has been considered as a potential target in new drug design.[37] The SARS-CoV-2 M^{pro} crystal structure, published in March 2020,[38] details the substrate-binding pocket (Fig. 6) and target amino acid residues for antiviral design. M^{pro} is a 3-domain (DI, DII, and DIII) autolytic endopeptidase and its

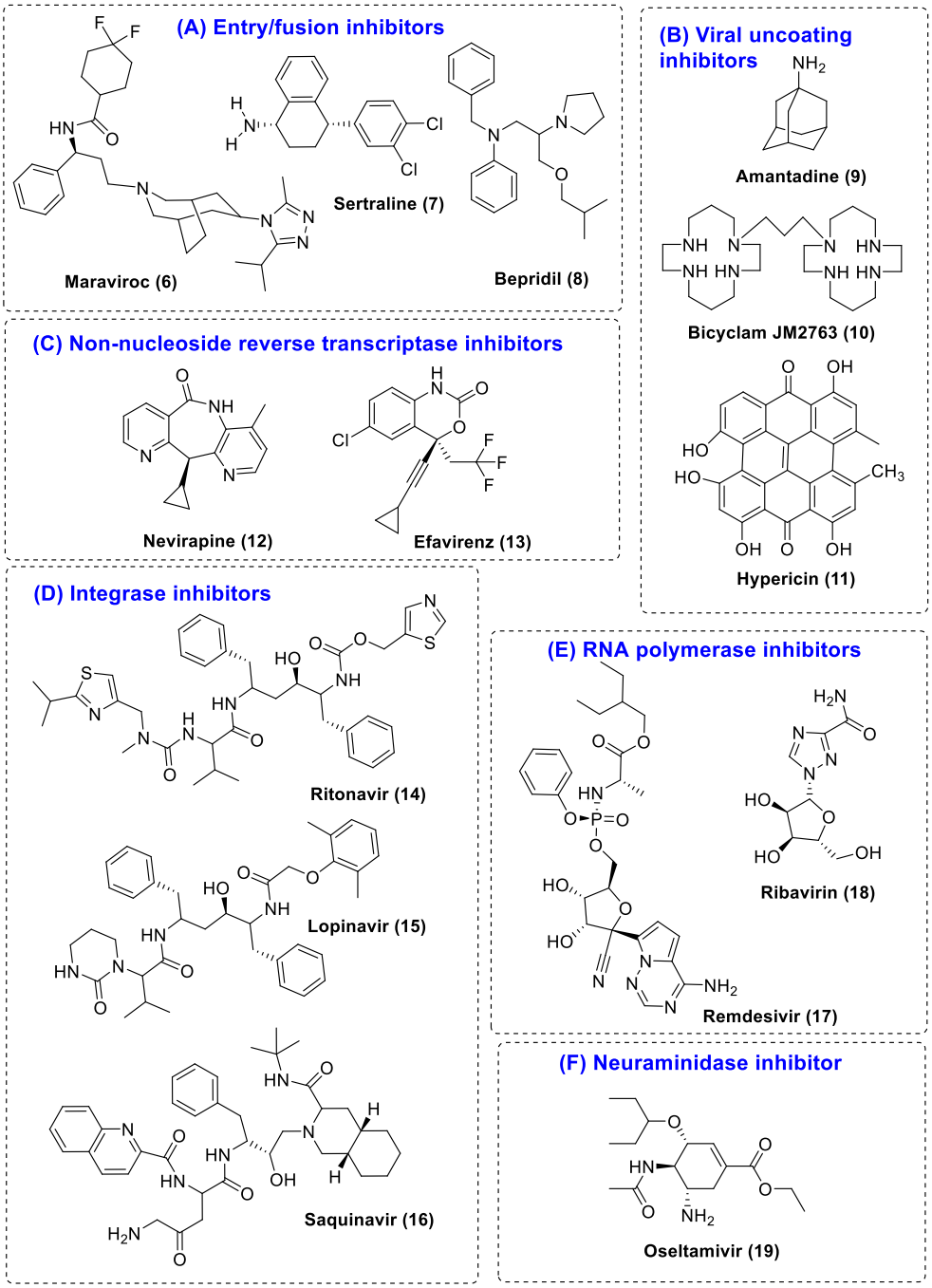

Fig. 7. Chemical structures of some current antiviral drugs and their targets. (A) **6**–**8**: maraviroc, bepridil and sertraline acting as entry/fusion inhibitors; (B) **9**–**11**: amantadine, bicyclan JM2763 and hypericin as viral uncoating inhibitors; (C) **12**–**13**: nevirapine and efavirenz as non-nucleoside reverse transcriptase inhibitors; (D) **14**–**16**: ritonavir, lopinavir and saquinavir as integrase inhibitors; (E) **17**–**18**: remdesivir and ribavirin nucleoside analogs which target the viral RNA polymerase; (F) **19**: oseltamivir as a neuraminidase inhibitor.

structure and sequence are conserved among identified coronaviruses, exclusively related to the Coronaviridae family.[34] The genome of SARS-CoV-2 has 82% similarity to its closest betacoronavirus, SARS–CoV, and M^{pro} holds 96% sequence identity. DI and DII hold the chymotrypsin catalytic gorge, including His41 and Cys145 residues, while DIII N–terminal residues promote homodimerization.[38] In contrast to SARS-CoV M^{pro}, SARS-CoV-2's M^{pro} homodimer does not present a hydrophobic interaction between DIII homodimers, due to a substitution of Thr285 and Ile286 residues to alanine and leucine, respectively, enhancing proteolytic activity.[38] At the same time, these SARS-CoV-2 peculiarities create prominent targets in drug design and development, possibly helping COVID-19 combat.

5. Main classes of antiviral drugs and their potential application to SARS-CoV-2 inhibition

From 1963 to 2016, 90 drugs were formally approved for the treatment of human viruses, eleven of which had broad coverage. Eight antiviral drug classes act on viral metabolic pathways: entry inhibitors, nucleoside and non-nucleoside reverse transcriptase, protease, integrase, acyclic nucleoside phosphonate, pyrophosphate, and 5–substituted 2′-deoxyuridine analogs. Some drugs target more specific proteins in the cells, such as HCV acyclic guanosine analogs, NS5A/NS5B, neuraminidase, and polymerase inhibitors. Other strategies do not directly target viral proteins, such as interferon immune stimulators, oligonucleotide analogs, and antimitotic inhibitors.[39]

The first class of inhibitors blocks the early stages of viral infection

during the entry phase, inhibiting the binding of glycoproteins of the viral envelope to host protein receptors. For example, a drug named BMS–378806 (Bristol-Myers Squibb), for human oral administration, is active against HIV-resistant strains, and against viruses with both CCR5 and CXCR4 coreceptors.[40] A closely related pyridine-derivative GSK3684934/BMS-663068 (Fostemsavir, GlakoSmithKline - GSK) was submitted to FDA approval. Entry/fusion inhibitors, available for HIV treatment, act at different points of virus penetration, such as the azabicyclic maraviroc (**6**) (Fig.7) (known as Celsentri in Europe, and Selzentry in the EUA, Pfizer Manufacturing), an antagonist of the HIV CCR5 coreceptor.[41] The peptide Enfuvirtide (T20, Fuzeon, Roche Diagnostics) acts by binding to the transmembrane HR1 region of HIV glycoprotein 41 (gp41), preventing the conformational change of gp41, which is required to complete the fusion process. Sertraline (**7**) (Zolof, Pfizer), a selective serotonin reuptake inhibitor, and bepridil (**8**) (Vascor, Ortho-McNeil-Janssen Pharmaceuticals), a calcium channel blocker, inhibit Ebola Virus (EBOV) entry.[42]

The second class of antivirals involves inhibitors of viral genome release (inhibitors of viral uncoating). The first drug approved in this class was amantadine (**9**), followed by rimantadine and derivatives, but the widespread viral resistance prevented their further utilization.[43] A bicyclam named JM2763 (**10**) and the natural products hypericin (**11**) and pseudohypericin act in the uncoating process.[44]

Nucleoside and non-nucleoside reverse transcriptase inhibitors, which interfere with the multiplication of the viral genome, are the third and fourth classes of antiviral drugs. After the virus enters the host cell, it begins to copy itself. Retroviruses need to copy their RNA into DNA, a process called reverse transcription, mediated by a reverse transcriptase enzyme, that can be inhibited by nucleoside-resembling molecules, called nucleosides reverse and non-nucleoside reverse transcriptase inhibitors.[45] The late are remarkably effective antivirals and, currently, there are six drugs approved by the FDA commercially available: nevirapine (**12**) (Viramune, Boehringer Ingelheim), delavirdine (Rescriptor, Vii Healthcare), efavirenz (**13**) (Fig. 7) (Stocrin, MerckSharp&Dohme; Sustiva, Bristol-Myers Squibb; Atripla, Gilead Sciences), etravirine (Intelence, Janssen Pharmaceutical), rilpivirine (Edurant, Janssen Pharmaceutical), and doravirine (Pifeltro, MerckSharp&Dohme).[45]

Integrase inhibitors and protease inhibitors are antiviral drugs that are mainly directed toward enzymes essential for viral replication.[46] Viral integrases allow the insertion of proviral DNA into the host genome, whereas proteases are involved in the editing of the viral protein arsenal. Three integrase inhibitors, ritonavir (**14**) (Norvir, Abbott Laboratories), lopinavir (**15**) (Fig. 7) (Kaletra, Abbott C4), and saquinavir (**16**) (Fortovase, Roche) are currently approved to be used alone or in combination with other drugs, in highly active antiretroviral therapy (HAART).[47] These drugs are protease inhibitors effective against MERS-CoV and HIV–1.[48] Viral mutations are associated with resistance to HIV protease drugs, and they have been frequently used with other drugs in HAART to target multiple stages of the virus life cycle. Ritonavir and ritonavir/lopinavir associations are indicated in more than 90 clinical trials for COVID-19 treatment, according to the World Health Organization's International Clinical Trials Registry Platform (WHO ICTRP) and ClinicalTrails.gov, maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH) (USA) (2021). Seven protease inhibitors have been approved for clinical use. Telaprevir (Incivo, Janssen-Cilag Pharmaceutica) and simeprevir (Olysio, Janssen-Cilag Pharmaceutica), although the most expensive ones, presented the better response rates.[44] Simeprevir showed great potential to inhibit the enzyme SARS-CoV-2 $3CL^{pro}$ in computational screening.[49] Ritonavir (**14**) and saquinavir (**16**) presented high binding energies for SARS-CoV-2 M^{pro}.[50] Mugisha et al.[51] demonstrated that E64D (inhibitor of endosomal protease cathepsin B and L) and apilimod (endosomal trafficking inhibitor) significantly reduced SARS-CoV-2 RNA in infected cell cultures, while amprenavir (an HIV-specific protease inhibitor) had a minor effect on the virus.

Nucleosides and nucleotides are other targets for the design of antiviral drugs, such as the adenosine analog GS-5734 (Remdesivir (**17**), Gilead Sciences) (Fig. 7), active against SARS-CoV and MERS-CoV.[52] This monophosphoramidate prodrug inhibits, *in vitro* and *in vivo*, the nonstructural protein 12 RNA-dependent RNA polymerase (RdRp), an essential part of the CoV replication-transcription complex.[52] Therefore, RdRp is another potential drug target for SARS-CoV-2, as Remdesivir (**17**) is capable of interact with SARS-CoV-2 main protease by means of a stable covalent bond.[50] Remdesivir is cited in more than hundred clinical studies for COVID-19 application, on WHO ICTRP and ClinicalTrails.gov platforms (2021).

Protease inhibitors are also the main targets of HCV infection. For instance, ribavirin (**18**) (Copegus, Roche) targets viral RNA polymerase, and is effective against five viruses: RSV, DENV, CHIKV, HCV, and IAV. Seven HCV NS3/4A protease compounds have been approved for clinical use, and telaprevir (INCIVO, Janssen-Cliag Pharmaceutica) and simeprevir (OLYSIO, Janssen-Cliag Pharmaceutica) have been reported to have better response rates, although they are more expensive.[44,53] Oseltamivir (**19**) (Tamiflu, Gilead Sciences), zanamivir (Relenza, GSK), and peramivir (Rapivab, Biocryst Pharmaceuticals) target viral neuraminidases or mRNA synthesis, and *in silico* studies demonstrated the docking of oseltamivir (**19**) to SARS-CoV-2 main protease.[54] Ribavirin (**18**) participates in six ongoing phase 2 or 3 clinical trials, and oseltamivir in seven clinical studies registered on ClinicalTrails.org (2021).

Most of the aforementioned compounds are synthetic prescription drugs studied in a repurposing approach, since the toxicity, side effects, and industrial production are already known. However, the limited structural diversity of synthetic compounds may be a drawback of this process. Considering the success of natural products as new lead drugs, the screening of these metabolites can increase the chances of developing effective antiviral drugs, taking into consideration the remarkable structural diversity of natural compounds, especially those produced by fungi.[55]

6. Diversity and structural complexity of fungal metabolites as models for new antiviral products

Antiviral fungal metabolites bearing complex chemical structures and novel skeletons from mixed biosynthesis, often considered rare compounds, including protease inhibitors, are well documented and can be further explored to develop potential SARS-CoV-2 inhibitors.[37] Whereas the terrestrial environment is the traditional source of fungi, deep-sea has been a rewarding source of biotechnologically promising fungi, such as some meroterpenoids (diterpene/polyketide hybrids). One of them, brevione F (**20**), which exhibits a singular α-pyrone pentacyclic basic carbon framework, inhibits HIV-1 *in vitro* replication in the human leukemia T cell line C8166.[56] Despite the complex chemical structures of metabolites of the brevione class, usually bearing six stereocenters, enantiocontrolled total synthesis of brevione-related derivatives such as brevione C (**21**) have been reported,[57] broadening the scope of potential antiviral drugs of compounds from this class. The heterocyclic ether present in the skeleton of brevione metabolites is an important bioisoster of peptide bonds in the development of protease inhibitors. The strong hydrogen bonds formed with the oxygen in the O-heterocycle ring enhance the interaction of these metabolites with the enzyme receptors of drug-resistant viral strains. Amprenavir and darunavir are FDA-approved anti-HIV drugs containing O-heterocycles, demonstrating the role of this moiety in the development of antiviral drugs.[58,59]

Cladosin C (**22**), a polyketide isolated from *Cladosporium sphaerospermum* (Ascomycota), sheds light on a novel class of tetramic acids, possibly formed by the action of a rare aminotransferase domain present in the polyketide synthase gene of this fungus. Cladosin C showed *in vitro* activity against the cytopathic effect of IAV H1N1.[60] Convergent total synthesis of cladosin C, applied to other members of the cladosin family, was reported.[61] Basidiomycota fungi also revealed promising antiviral substances, such as rhodatin (**23**), a novel spirospiroketal pentacyclic

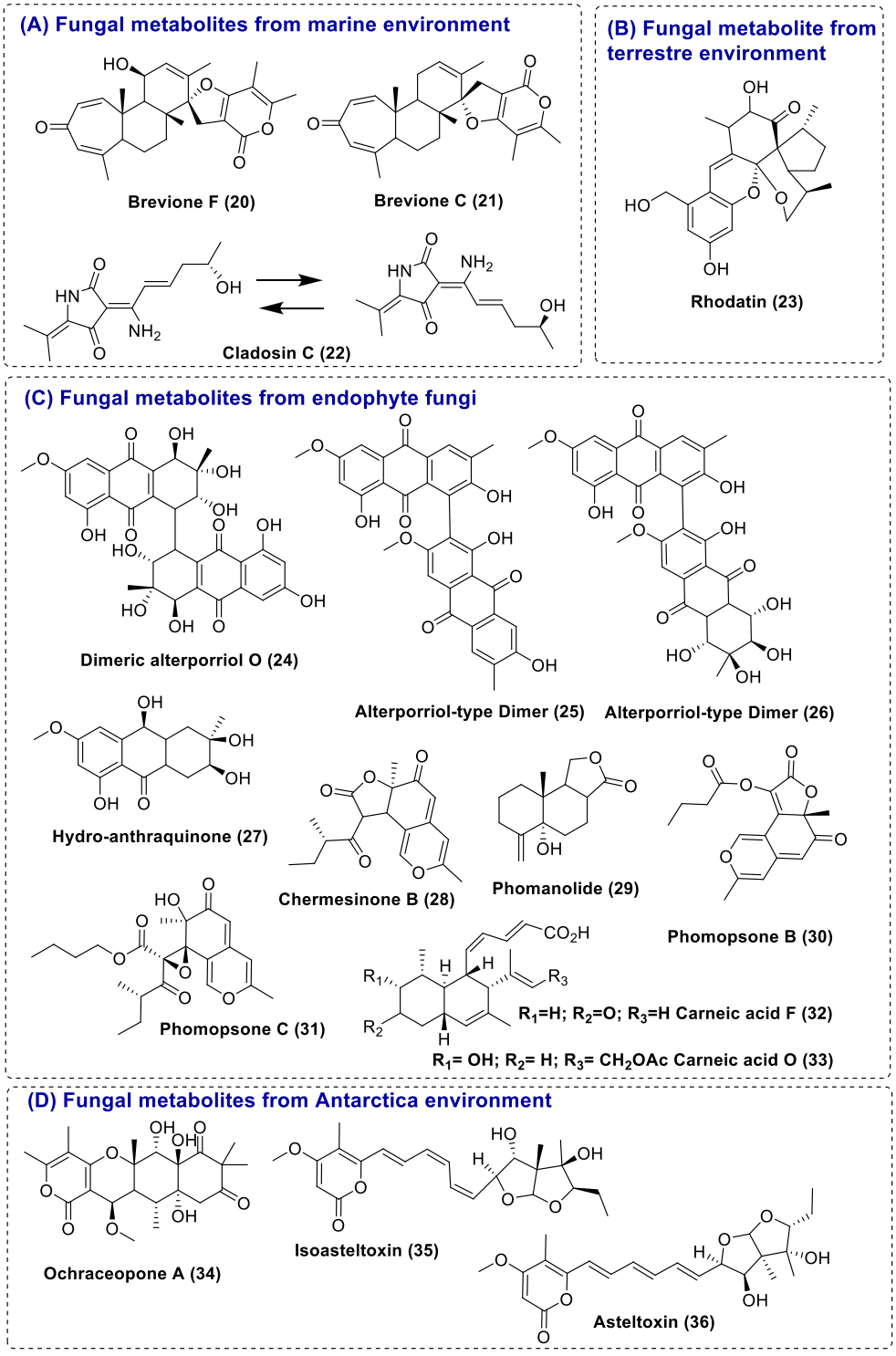

Fig. 8. Chemical structures of fungal metabolites (**20**–**36**) reported as antiviral agents.

meroterpene, isolated from the pink mushroom *Rhodotus palmatus*, which is highly active against *in vitro* HCV-infected human liver cells.[62]

Endophytic fungi are another niche that has gained much attention because these organisms can acquire characteristics and uniqueness from host plant biosynthesis. Dimeric anthraquinone alterporriol O (**24**), which features a new C4-C4′ linkage, was isolated from an endophytic strain of *Alternaria* sp., along with two other alterporriol dimers (**25** and **26**).[63] The dimers and a hydro-anthraquinone monomer (**27**) demonstrated *in vitro* activity against porcine reproductive and respiratory syndrome virus (PRRSV) replication in CRL 11,171 cells. Chermesinone B (**28**), an azaphilone recovered from *Nigrospora* sp., was active against IAV H1N1 in cytopathic inhibition assays.[64] An endophytic strain of *Phoma* sp. (YE3135) produced phomanolide (**29**), a new rare 14-nordrimane-type sesquiterpenoid, which was able to inhibit the *in vitro* cytopathic effect of IAV.[65] *In vitro* antiviral activity against HIV-1 was reported for phomopsones B (**30**) and C (**31**), new azaphilones with pyranoquinoid core structures, isolated from *Phomopsis* sp. CGMCC No.5416.[66] Carneic acids F and O (**32** and **33**), isolated from another

Table 2
Examples of fungal metabolites, activity against DENV, HV, H1N1, HIV, and ZIKV, and fermentation conditions.

Fungus name [Origin]	Bioactive metabolite	Antiviral activity [Virus strain]	Culture medium composition [Fermentation details]
Dengue Virus (DENV)			
Dichotomomyces cejpii F31-1 [Marine inner tissue of the soft coral *Lobophytum crassum*, P. R. China][71]	Scequinadoline A (**37**)	EC_{50} 4.73 μM [DENV 2 strain 16,681]	Sea salt (30 $g.L^{-1}$, glucose (20 $g.L^{-1}$), peptone (5 $g.L^{-1}$), yeast extract, L-Phe and D, L-Trp (2 $g.L^{-1}$ each) [pH 7.5, 25 °C, 60 days]
Penicillium sp. FKI-7127 [Soil around the root of *Angelica keiskei* collected in Kouzu Island, Japan][70]	Brefeldin A (**38**)	IC_{50} 54.6 ± 0.9 nM [DENV2 strain 00st-22A]	Soluble starch 3%, glycerol 1.0%, soybean meal 2%, dry yeast, KCl, $CaCO_3$, KH_2PO_4, $MgSO_4{\cdot}7H_2O$, and quercetin dihydrate (0.03–0.3%) [6 days; other details not informed]
Phomopsis sp. SNB-LAP1-7–32 [Leaves of *Diospyros carbonaria*, Saint Elie, French Guiana][67]	Carneic acid F (**32**) Carneic acid O (**33**)	IC_{50} 11.8 ± 0.9 μM IC_{50} 13.6 ± 1.5 μM	PDA [26 °C, 15 days]
Hepatitis C Virus (HCV)			
Scedosporium apiospermum 2014F41-1 [Marine soft coral *Lobophytum crassum* collected from Hainan Sanya National Coral Reef Reserve, P. R. China][72]	Scedapin C (**39**)	EC_{50} 110.35 μM [HCV genotypes 2b, J8cc]	Glucose (10 $g.L^{-1}$), peptone (5 $g.L^{-1}$), yeast extract (2 $g.L^{-1}$), L-Phe, L-Trp, D,L-Met, L–Lys, L-Thr (1–2 $g.L^{-1}$), sea salt (22 $g.L^{-1}$)
	Scequinadoline D (**40**)	EC_{50} 128.60 μM [HCV genotypes 2b, J8cc]	[pH 7.5, 28 °C, 40 days]
Influenza Virus (IAV)			
Aspergillus sp. SCSIO XWS02F40 [Sponge *Callyspongia* sp., collected from the sea area near Xuwen County, China][73]	Asteltoxin E (**41**)	EC_{50} 3.5 ± 1.3 μM [Influenza H1N1] EC_{50}6.2 ± 0.08 μM [Influenza H3N2]	Rice (200 g), sea salt (2.5 g) 200 mL water [25 °C, 30 days]
	Asteltoxin F (**42**)	EC_{50} 8.9 ± 0.3 μM [Influenza H3N2]	
Phoma sp. strain YE3135 [roots of *Aconitum vilmorinianum*][65]	Phomanolide (**29**)	IC_{50} 2.96 ± 0.64 μg/mL [A/Puerto Rico/8/34]	PDB medium [28 °C, 11 days, 185 rpm]
Nigrospora sp. YE3033 [roots of *Aconitum carmichaelii* collected in Lijiang County, P. R. China][64]	6-O-Demethyl-4-dehydroxyaltersolanol A (**43**)	IC_{50} 2.59 ± 1.22 μg/mL [A/Puerto Rico/8/34]	PDB medium [28 ± 1 °C, 7 days, 200 rpm]
	4-Dehydroxyaltersolanol A (**44**)	IC_{50} 8.35 ± 1.41 μg/mL [A/Puerto Rico/8/34]	
	Altersolanol B (**45**)	IC_{50} 7.82 ± 1.86 μg/mL [A/Puerto Rico/8/34]	
	Chermesinone B (**28**)	IC_{50} 0.80 ± 0.29 μg/mL [A/Puerto Rico/8/34]	
Aspergillus sydowii SCSIO41301 [sponge *Phakellia fusca*, collected from the Xisha Islands, China][74]	2-Hydroxy-1-(hydroxymethyl)-8-methoxy-3-methyl-9H-xanthen-9-one (**46**)	IC_{50} 4.70 ± 1.11 μM [Puerto Rico/8/34]	Mannitol and maltose (20 $g.L^{-1}$ each), glucose and monosodium glutamate (10 $g.L^{-1}$ each), KH_2PO_4(0.5 $g.L^{-1}$), $MgSO_4{\cdot}7H_2O$ (0.3 $g.L^{-1}$), yeast extract (3 $g.L^{-1}$), tap water [pH 7.5, 28 °C, 35 days, static]
	2-Hydroxy-1-(hydroxymethyl)-7,8-dimethoxy-3-methyl-9H-xanthen-9-one (**47**)	IC_{50} 2.17 ± 1.39 μM [Puerto Rico/8/34]	
Human Immunodeficiency Virus (HIV)			
Phomopsis sp. CGMCC No.5416 [fresh stems of *Achyranthes bidentata* collected from Nan Ling County, China][66]	Phomopsone A (**48**)	IC_{50} 0.5 μM [HIV-1]	Rice (80 g 100 mL^{-1} water) [28 °C, 30 days]
Penicillium sp. IMB17-046 [marine sediments collected from a mangrove swamp in Sanya, China][69]	3β-hydroxyergosta-8,14,24(28)-trien-7-one (**49**)	IC_{50} 3.5 ± 0.8 μM [HIV-1]	Rice (100 g), peptone (0.3 g 100 mL^{-1} water [28 °C, 4 weeks]
	Truncateol P (**50**)		

(*continued on next page*)

Table 2 (*continued*)

Fungus name [Origin]	Bioactive metabolite	Antiviral activity [Virus strain]	Culture medium composition [Fermentation details]
Truncatella angustata XSB-01–43 [Reef-finger sponge *Amphimedon* sp. collected in Yongxing Island, China][75]		IC_{50} 16.1 ± 0.7 μM [HIV-1]	Rice (80 g 100 mL^{-1} water [25 °C, 40 days]
Zika Virus (ZIKV)			
Fusarium sp. L1 [sea star *Acahnthaster planci*, Xisha Islands, China][76]	Fusaindoterpene B (**51**) 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione (**52**)	EC_{50} 7.5 μM EC_{50} 4.2 μM EC_{50} 5.0 μM	GPY liquid medium [28 °C, 40 days]
Colispora cavincola[77,78]	Cavinafungin (**53**)	IC_{50} 150.0 nM	Supermalt medium (malt extract 50 g L^{-1}, yeast extract 10 g.L^{-1}, $FeSO_4{\cdot}7H_2O$ 20 mg.L^{-1}, $ZnSO_4{\cdot}7H_2O$ 20 mg.L^{-1}) [22 °C, 21 days, static]

Phomopsis species (SNB-LAP1-7–32), exhibited significant inhibition of DENV-2 polymerase. The activity of carneic acid F (**32**) was attributed to the β-hydroxyl group present in its structure.[67]

The screening of fungi from niches such as Antarctica is supported by the metabolic differentiation in fungal biosynthesis necessary for survival under drastic climatic conditions. This behavior was observed for the Antarctic species *Aspergillus ochraceopetaliformis* (SCSIO 05702), which produces ochraceopone A (**34**), a linear tetracyclic α–pyrone merosesquiterpenoid with a new skeleton, along with isoasteltoxin (**35**) and asteltoxin (**36**). These compounds effectively inhibited the cytopathic *in vitro* effect of IAV H1N1 and H3N2 strains.[68] The structures of metabolites **20**–**36** are shown in Fig. 8.

7. Strategies on the search of new antiviral metabolites from fungi

The adaptive ability of fungi to different fermentation protocols, upon alteration of parameters such as carbon and nitrogen sources, availability of macro and micronutrients, pH, fermentation length, and temperature variation, can be exploited in the production of new antiviral metabolites. Rice is frequently reported as a substrate for the production of fungal metabolites [69] (Table 2), although reproducibility in rice-based culture medium should be carefully evaluated, considering commercial variation. The cultivation length can be as short as six[70] or more than seventy days.[71] Table 2 presents the profiles of antiviral activity and fermentative conditions to produce fungal metabolites such as scequinadilone A (**37**) and brefeldin A (**38**), active against DENV, scedapin C (**39**), scequinadoline D (**40**) active against HVC, asteltoxins E and F (**41** and **42**), altersolanols (**43**–**45**) and xanthenones (**46**–**47**) (IAV inhibitors). Metabolites active against HIV (**48**–**50**) and Zika Virus (ZIKV) (**51**–**53**) are also shown. The structures of the metabolites **37**–**53** are shown in Fig. 9.

Measurement of medium depletion can determine the fermentation stop-point, an approach used by Narmani et al.[79] during the recovery of prenylated *p*-terphenyl quinine metabolites from *Cytospora* sp. However, fermentation length does not follow a restrictive unique rule regarding productivity, as exemplified by experiments with *Scedosporium apiospermum* (40 days; 0.22 g l^{-1} yield),[72] *Phoma* sp. (11 days; 1.1 g L^{-1} yield),[74] and *Simplicillium obclavatum* (30 days; about the same yield, 1.09 g L^{-1}).[80]

In most studies cited herein, metabolite isolation usually started using silica gel vacuum liquid chromatography, followed by a Sephadex LH20 column,[71] preparative Reversed Phase (RP)-C18 High-Performance Liquid Chromatography (HPLC),[71,75] and RP-18 silica gel.[74] Direct loading of aqueous extract on a Sep–Pak plus ODS cartridge may be incorporated in the purification protocol,[70] as well as the use of amberlite XAD-16 polymeric resin.[79] XAD-16 resin (non-ionic polystyrene-divinylbenzene) is highly stable in both acidic and basic solutions, and selectively retains substances in the column without exhaustion of the column material.[81] This process might be used as a cleanup step, before chromatographic separation.

Nevertheless, the purification of fungal metabolites in research laboratories for antiviral screening can become a game of patience. During the development of new drugs, low yields can be overcome using bench-size bioreactors and genetic manipulation.[82] Detection of the major compounds present in crude extracts, before the isolation step, is useful to direct the efforts, optimize time-consuming purification processes, and avoid re-isolation of known compounds. Nuclear Magnetic Resonance (NMR)-directed isolation is very effective in targeting specific classes of compounds in crude extracts or fractions. In this approach, the isolation step is monitored by NMR, and only extract/fractions with chemical shifts related to the target compounds are further purified or assayed, as successfully demonstrated during the isolation of allenyl (**54**–**56**) and alkynyl truncateols O (**57**) and P (**50**) from *Truncatella angustata*.[75] Metabolome analysis by Liquid Chromatography coupled to High-Resolution Mass Spectrometry (LC–HRMS)[80], Liquid Chromatography-Electrospray Ionization-Quadrupole-Time of Flight-Mass Spectrometry (LC–ESI–Q–TOF–MS)[83] can quickly map secondary metabolites from molecular ions, with high accuracy and isotopic ratio measurements. Ultra-Performance Liquid Chromatography (UPLC) may speed the process, coupled with HRMS, providing both quantitative and structural information, achieving pgmL^{-1} sensitivity.[84] Nothias et al.[85] identified a "bioactive molecular networking," applying Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) to fractions obtained from bioactive extracts.

Biosynthesis-directed fermentation is an alternative approach for obtaining secondary metabolites. Huang et al.[72] supplementation of amino acids (L-tryptophan, L–phenylalanine, L-threonine, and D, L–methionine) to the culture medium led to the production of three formamides and 18 quinazoline-containing indole alkaloids. A metabolite bearing a rare pyrazinoquinazolinedione, scedapin C (**39**) and a new scequinadoline alkaloid (**40**) isolated in that study inhibited HCV *in vitro*.

In the aforementioned examples, the initial step of the prospective studies to detect antiviral metabolites from fungi usually consisted of *in vitro* screening of potential leads, starting from the assessment of cytotoxic and side effects on host cells in culture assays. After preliminary results, the selected molecules should be targeted by mechanism-directed assays. Standardized protocols and commercial kits for biochemical or cell-based assays are important resources for determining viral replication, cell viability and cytotoxicity, or specific metabolic and signaling pathways, ensuring reproducibility and allowing bench or high-throughput screening of potential antiviral compounds.[86] However, *in vitro* assays usually have limitations in predicting the pharmacokinetic and pharmacodynamic mechanisms, as well as the potential *in vivo* adverse side effects elicited by drug candidates. Thus, in the last decade, new *in vitro* techniques for pre-clinical studies have been developed, such as three-dimensional cell cultures of primary or established cell line origin, and *ex-vivo* models such as organotypic culture assays. These organoids and organ-on-a-chip are innovative alternatives to mimic *in vivo* conditions on a small scale, allowing the study of virus infection mechanisms and the evaluation of antiviral drug candidates in

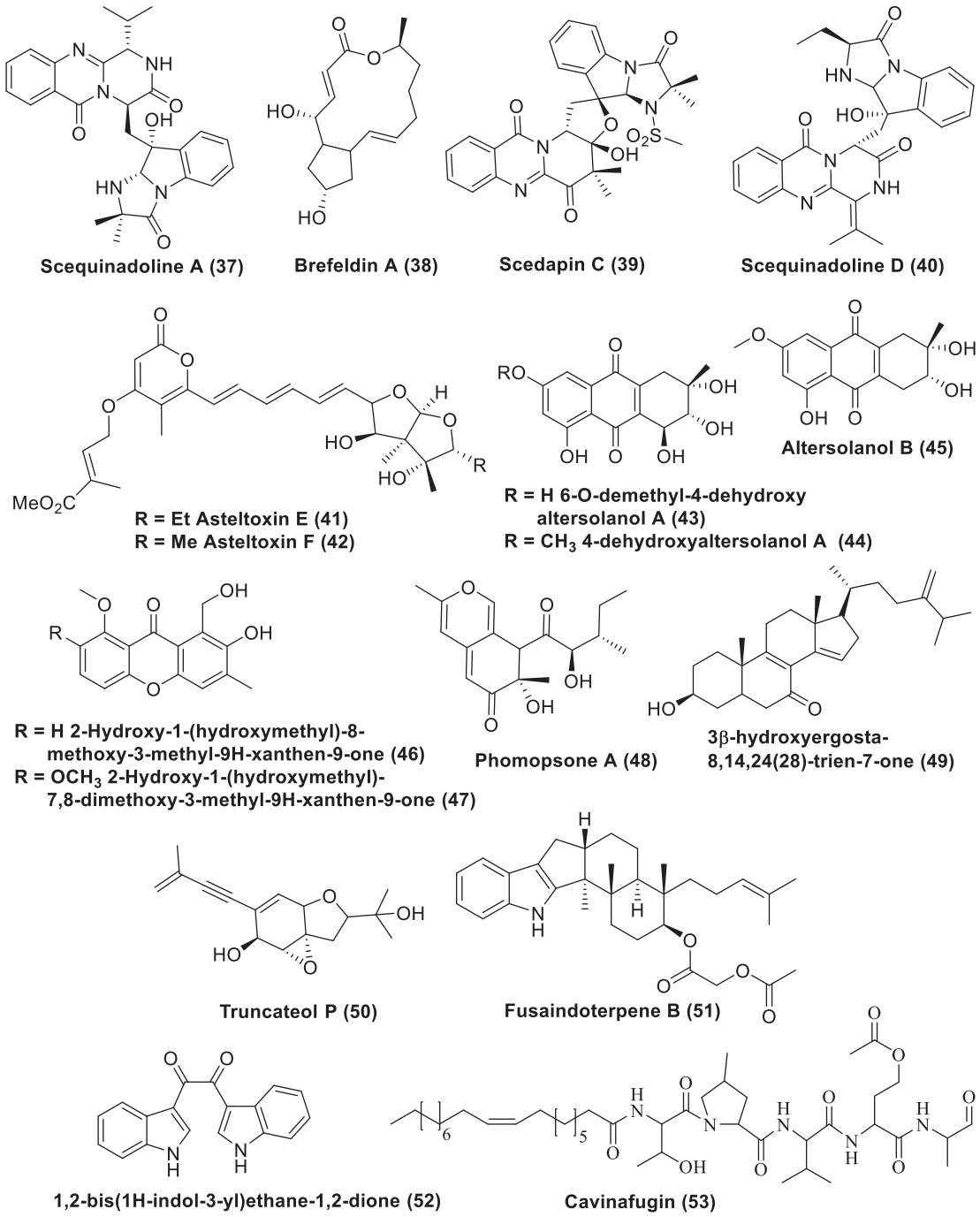

Fig. 9. Chemical structures of antiviral fungal metabolites **37**–**53.**

complex biological structures.[87,88]

The quantity of viral proteins and reduction of viral nucleic acids in infected cells have been measured in assays against non-cytopathic viruses. Polymerases are essential for all viruses and are excellent targets for antiviral therapies; therefore, quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) has been the preferred method for monitoring the activity of DNA polymerases (cellular or viral) in the presence of inhibitors,[89] and has become a standard model for screening viral RNA polymerase inhibitors.[90] Quantitative detection can be achieved using a wide variety of sequence-specific probes or non-specific fluorescent dyes that bind to the genetic material. Mugisha et al.[51] proposed a simplified and efficient method to detect viral RNA in cell culture supernatants, to reduce the costs of qRT-PCR used to monitor the response of SARS-CoV-2 to potential antivirals.

Additionally, computer-aided drug design has been widely utilized in the search for antiviral agents, because it is a reliable, fast, sustainable, and cheap approach compared to wet-lab testing. Tridimensional molecular models of candidate molecules and/or molecular targets can be built and studied *in silico* under different conditions, and the results are statistically processed. This strategy can be directed to the receptor (structure-based drug design) or ligand (ligand-based drug design) depending on a series of factors and data availability.[91] Many studies have used structure–activity relationship (SAR) tools to identify potentially bioactive metabolites and to plan the synthesis of derivatives bearing antiviral pharmacophoric groups. This approach led to the identification of exocyclic double bond and oxidation on specific groups as key structural features related to the inhibition of HIV-1 cellular infection by eight armochaetoglobins isolated from the symbiotic fungus

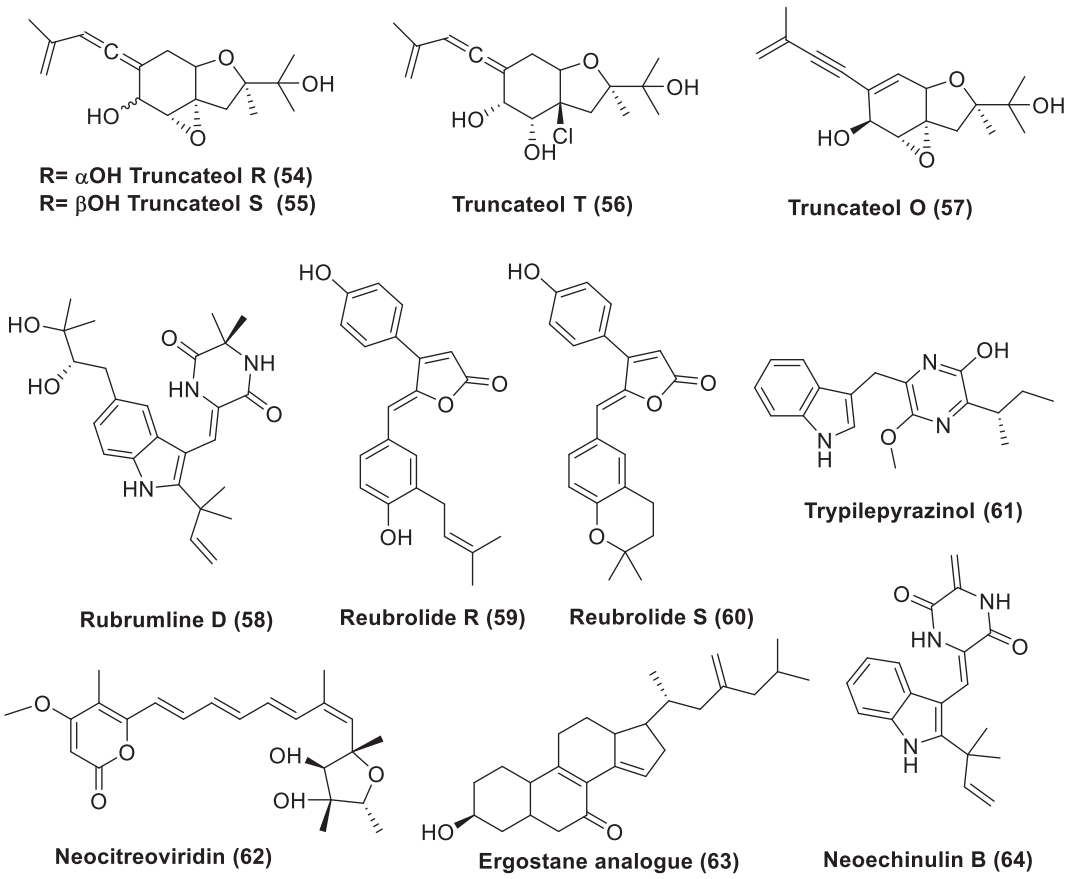

Fig. 10. Chemical structures of antiviral fungal metabolites **54**–**64.**

Chaetomium globosum TW1-1.[92] Likewise, the inhibit the cytopathic effect of IAV H1N1 (A/WSN/33 strain) was associated by SAR with specific substituents at the diketopiperazine moiety of rubrumline D (**58**) and other related metabolites, isolated from marine-derived *Eurotium rubrum* F33.[93] The positive effect of alkynyl groups in activity enhancement, reported for truncateols O and P (**57** and **50**) (Fig. 10), metabolites isolated from the fungi species *T. angustata*, were successfully pointed by SAR studies.[75] The low cytotoxicity of these truncateols compared to the positive control used in the work, efavirenz (**13**), an antiretroviral drug prescribed for patients with HIV-1, encourages further studies on this class of compounds.

SAR is also important for the synthesis of analogs, as shown in the stereoselective synthesis of reubrolides R and S (**59** and **60**), where SAR directed the introduction of a β-aryl substituent at a late stage of the synthesis, aiming at the preparation of antiviral compounds.[94] In the synthesis field, click-chemistry and modern fluorescence microscopy techniques together allow site-specific design of new molecules, based on the interaction of host cells and viral factors, and seems very promising in the near future.[95]

Multi-target and *in silico* screenings are advantageous over single strain-directed assays, as a fungal species can produce metabolites that are active against different viruses. For example, three compounds isolated from a marine-derived species of *Penicillium* sp. were screened to assess the relative inhibition of three viruses (HIV-1, IAV H1N1 and HCV). One of the compounds, trypilepyrazinol (**61**) demonstrated *in vitro* protective effects against HIV-1 and HCV. Trypilepyrazol is a new polyketide derivative that contains a pyrazine heterocycle, an important pharmacophore found in many bioactive drugs, as part of its structure. The other two compounds, (+)-neocitreoviridin (**62**) and a new ergostane analog (**63**) presented a different behavior, being active against IAV H1N1.[69] The different selectivities of metabolites **61**, **62**, and **63** were only observed because different viruses were included in the experimental protocol. On the other hand, some metabolites present broad antiviral activity, as reported for brefeldin A (**38**) (Fig. 9), isolated from *Penicillium* sp. FKI-7127, which selectively inhibited four Filipino patient-derived DENV strains and ZIKV (strain 976) once subjected to a multi-target antiviral assay.[70] The structures of metabolites **54**–**64** are shown in Fig. 10.

Resistance is a major problem that must be taken into consideration in antiviral screening to develop drugs that are effective also against resistant strains. In this context, the fungal metabolite neoechinulin B (**64**), produced by *E. rubrum* F33, was tested against IAV, a virus resistant to commercial drugs such as ribavirin (**18**). The fungal metabolite neoechinulin B (**64**) acts at the viral entry stage, binding to the viral hemagglutinin. As the interaction with sialic acid receptors on host cells is avoided, this compound does not promote significant drug resistance in clinical isolates.[93]

Overall, the chemical structures of antiviral drugs **6**–**19** are diversified enough to selectively target RNA viral proteins according to their respective mechanisms of action. Pharmacophore-based approaches are among the current protocols for repurposing drugs as SARS-CoV-2 inhibitors. Using this approach, compounds bearing indoloquinoline, benzimidazole, indolyl, and carbamate moieties were among the five top drugs with higher docking scores to inhibit viral M^{pro}.[96] In the same way, antiviral fungal metabolites also feature high chemical diversity, including heterocyclic diazo scequinadoline A (**37**), macrocycle brefeldin A (**38**), and anthraquinones (**44** and **45**). There are some similarities among the structures of the antiviral drugs (Fig. 7) and antiviral fungal metabolites (Figs. 8–11). Fungal metabolites chetomin (**3**), cladosin C (**22**), scequinadoline A and D (**37** and **40**), and trypilepyrazinol (**61**) present nitrogenated heterocycles in their structures, a moiety commonly found in the structure of antiviral drugs. In addition, the

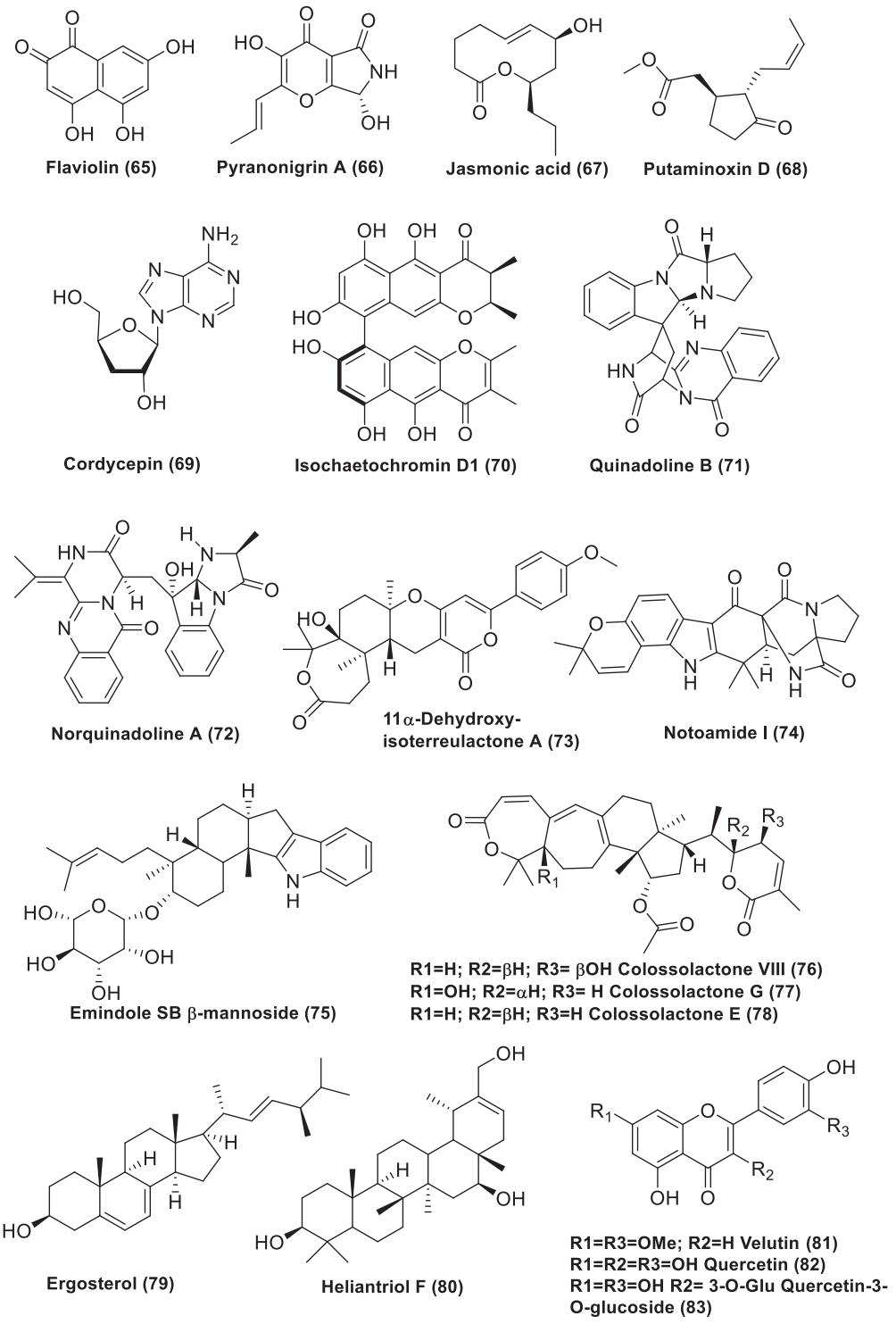

Fig. 11. Chemical structures of fungal metabolites **65**–**83** with potential SARS-CoV-2 antiviral effect.

indole moiety, an important pharmacophore targeted in the research for novel antiviral drugs,[97] is present in the structures of fusaindoterpene B (**51**), bisindol derivative (**52**), rubrumline D (**58**), tryplepyrazol (**61**), neoechinulin (**64**), and notoamide I (**74**). Some pharmacophore groups present in the structure of several of the metabolites herein discussed, such as azaphilone (compounds **28**, **30** and **31**), α-pyrone (compounds **20**, **21**, **34**–**36**, **41**, **42**, **62,** and **73**) and anthraquinone (**25**) rings have been suggested as possible inhibitors of SARS-CoV-2, considering the protease inhibition related to compounds with similar structures.[48]

All these antiviral metabolites biosynthesized by fungi, with unique molecular structures, are interesting molecules in the search for new antiviral candidates against SARS-CoV-2. In addition, the rational design of inhibitors of influenza virus replication[98] and HIV-1 protease inhibitors,[99] can be applied in the search for drug leads against SARS-CoV-2[100]. These are encouraging data in the ongoing search for new antiviral drugs since, despite the importance of vaccines, antiviral medicines are necessary to treat infected patients for symptom relief, reducing the hospitalization period.

Table 3
Fungi secondary metabolites with potential SARS-CoV-2 antiviral effect.

Metabolite	Source	Mechanism	Methodology
Flaviolin (**65**)[101] Pyranonigrin A (**66**)[102]	Exploratory library metadata	Inhibition of M^{pro} ($3CL^{pro}$), drug-like ADMET properties	*In silico* molecular docking analysis, dynamics simulation and ADMET predictions
Jasmonic acid (**67**) and Putaminoxin B (**68**)[108]	*Stagonospora cirsii* *Phoma putaminum*	Inhibition of M^{pro}, drug-like ADMET properties	*In silico* molecular docking analysis, dynamics simulation and ADMET predictions
Cordycepin (**69**)[107,109]	*Cordyceps militaris*	Binding affinity to S protein and M^{pro}, inhibition of poly (A) polymerase, action in multiple pathways	*In silico* molecular docking analysis, pharmacology network prediction, SAR
Scedapin C (**39**), Isochaetochromin D1(**70**),	Exploratory library metadata	Binding affinity to PL^{pro}, M^{pro}, RdRp, nsp15, and S binding domain	*In silico* molecular docking analysis, dynamics simulation, and ADMET predictions
Quinadoline B (**71**) Norquinadoline A (**72**), and 11α-dehydroxyiso-terreulactone A (**73**)[110]		Binding affinity to PL^{pro}, M^{pro}, RdRp, nsp15, and S binding domain; high gastrointestinal absorption, poor blood–brain barrier penetrability, druggability, non-toxic, non-carcinogenic, non-mutagenic	
Notoamide I (**74**) and Emindole SB β-mannoside (**75**)[100]	ChEMBL database	Inhibition of M^{pro} Non-toxic, non-carcinogenic, non-mutagenic	*In silico* QSAR, molecular docking analysis and ADMET predictions
Colossolactone VIII (**76**), Colossolactone G (**77**), Colossolactone E (**78**),	*Ganoderma colosum*	Binding affinity to M^{pro} Non-toxic, non-carcinogenic, non-mutagenic	*In silico* molecular docking analysis and ADMET predictions
Ergosterol (**79**),	*Auricularia polytricha, Flammulina velutipes, Lentinula edodes*		
Heliantriol F (**80**), and Velutin (**81**) [112]	*Lignosus rhinocerus* *Flammulina velutipes*		
Quercetin [a] (**82**)[111,116,117]	PubChem database	Affinity to M^{pro} and viral RNA polymerase,drug-like ADMET properties	*In silico* molecular docking analysis, ADMET predictions

S protein = SARS-CoV-2 spike protein; M^{pro} = SARS-CoV-2 main protease, also known as $3CL^{pro}$ = SARS-CoV-2 chymotrypsin-like protease; PL^{pro} = papain-like protease; $3CL^{pro}$ = SARS-CoV-2 chymotrypsin-like protease; RdRp = RNA-directed RNA polymerase; nsp15 = non-structural protein 15.
ADMET = absorption, distribution, metabolism, excretion, and toxicity; QSAR = quantitative structure–activity relationship; SAR = structure–activity relationship.
[a] Molecule on clinical trial, according to the databases available on the World Health Organization's International Clinical Trials Registry Platform (WHO ICTRP) and ClinicalTrails.gov, maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH) (USA) (accessed June 2021).

8. SARS-CoV-2 inhibitors based in known fungi metabolites

In the worldwide efforts to find an effective treatment for COVID–19, several FDA-approved drugs, from distinct natural sources or synthetically designed, are being evaluated for their interaction with SARS-CoV-2 proteins, using *in silico* strategies. Most *in silico* studies, as shown, use molecular docking, which allows the estimation of the activity of a target ligand at the receptor site, based on the most probable chemical interactions between them.[91,101,102] Docking programs have been improved to add new tools, aiming to increase the reliability of the process and, consequently, the chance of discovering potential drug leads. A combination of docking studies with the molecular dynamic approach, such as the super-computer-based drug discovery pipeline, was reported by Acharya et al.[103]

Molecular docking has already revealed the high affinity of oseltamivir (**19**), delavirdine, ritonavir (**14**), saquinavir (**16**) and remdesivir (**17**) for SARS-CoV-2 main protease, achieved by a covalent bond to Cys145 and variable H-bonds. Additional molecular dynamics analysis demonstrated a reduction in M^{pro} structural flexibility induced by **14**, **16** and **17**, indicating a possible impairment of its biological function.[50] The high affinity of etravirine, rilpivirine, and nevirapine (**12**) for the SARS-CoV-2 protease active site was also revealed by molecular docking studies, and nevirapine (**12**) presented a higher IC_{50} (half-maximal inhibitory concentration) for SARS-CoV-2 than for HIV protease.[54] Docking simulations directed to drug repurposing spare time and resources, since prescription drugs have already passed the initial clinical and toxicity trials.[50,54,104–107]

Likewise, a number of fungal natural compounds have been identified as interesting starting points for the development of COVID-19 therapeutic agents, as indicated by computational studies and preliminary *in vitro* assays.[100–102,106–113] Based on their similarities to the non-nucleoside reverse transcriptase inhibitor efavirenz (**13**), considered a repurposing drug candidate for COVID-19 treatment,[23] the FDA-approved metabolite podophyllotoxin isolated from endophytic fungi,[114] lovastatin (a well-known fungal metabolite) and its derivative simvastatin were evaluated for their interaction with SARS-CoV-2 proteins, using virtual screening, molecular docking, and density functional theory, with promising results.[106]

Table 3 reports complementary *in silico* analysis made to determine the affinity of some molecules deposited in databases and fungal metabolites for the main proteins of SARS-CoV-2, aiming at determining their potential side effects as antiviral drugs. Their corresponding chemical structures are presented in Figs. 9 and 11.

Using molecular docking, dynamics simulations and ADMET analysis to screen a hundred of fungi secondary metabolites, Rao et al.[101,102] revealed flaviolin (**65**) and pyranonigrin A (**66**) as drug candidates against SARS-CoV–2, considering their ability to bind and inhibit M^{pro}, and their drug-likeness ADMET properties, especially low toxicity, when compared with the synthetic compound N3 (PubChem CID 6323191) used as positive control (Table 3, Fig. 11). The same approach was applied in another study, with a larger number of metabolites from different taxa, pointing to three other fungal compounds, jasmonic acid (**67**) and putaminoxin D (**68**), as potential inhibitors of M^{pro}, with favorable ADMET properties.[108]

Cordycepin (**69**) is a secondary metabolite produced by *Cordyceps militaris*, with a broad spectrum of biological activities, including antiviral action.[115] Molecular interaction simulations revealed its high binding affinity to both SARS–CoV–2 M^{pro} and spike protein binding sites.[107,109] Moreover, this molecule has a remarkable similarity to adenosine, indicating an additional role in inhibiting the poly(A) polymerase, which is essential for 3′–polyadenylation of viral RNAs like SARS-CoV-2.[107] Pharmacology network predictions also reinforce the potential role of cordycepin (**69**) in multiple biological pathways associated with viral infections, signaling it as a repurposing drug candidate for COVID–19 treatment.[107,109]

Quimque et al.[110], using molecular docking and dynamic simulations

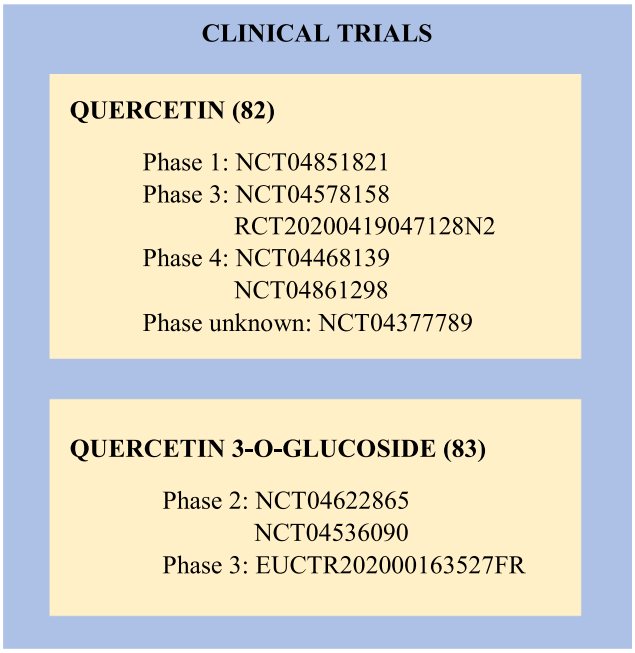

Fig. 12. Clinical trials with quercetin (**82**) or with its 3-O-glucoside (**83**), recorded on WHO ICTRP and ClinicalTrials.gov (July 2021).

to screen 97 fungal metabolites previously related to antiviral effects, revealed five multitarget molecules, scedapin C (**39**), isochaetochromin D1 (**70**), quinadoline B (**71**), norquinadoline A (**72**), and 11α–dehydroxyiso-terreulactone A (**73**), with dynamic stable binding affinities to SARS–CoV–2 proteases PL^{pro} and $3CL^{pro}$, RNA-directed RNA polymerase (RdRp), non-structural protein 15 (nsp15), and S protein. These metabolites were then submitted to *in silico* ADMET predictions, indicating that quinadoline B (**71**) is the most promising, according to its pharmacokinetic profile, especially oral bioavailability, high drug-likeness, and absence of toxicity.[110]

Using similar *in silico* approaches, several other fungal secondary metabolites were indicated as promising drug-like leads against SARS-CoV-2 M^{pro}. Notoamide I (**74**) and emindole SB β-mannoside (**75**) were selected from a panel of 494 marine natural substances, pre-evaluated by a QSAR classification model, and further by molecular docking and ADMET predictions.[100] Among the 36 metabolites derived from edible and medicinal mushrooms, colossolactone VIII (**76**), colossolactone G (**77**), colossolactone E (**78**), ergosterol (**79**), heliantriol F (**80**), and velutin (**81**) were emphasized as good drug candidates as they did not presented relevant toxic, carcinogenic, or mutagenic side effects.[112]

Quercetin (**82**) is also a promising candidate for the development of drugs for COVID-19 treatment. According to *in silico* studies, the compound demonstrated high affinity for the active site of $3CL^{pro}$, as well as for the active site and the NiRAN subdomain of SARS-CoV-2 RNA-polymerase, potential targets to viral inhibition.[111] Quercetin (**82**) presents important advantages in drug development, as its pharmacokinetic and ADMET properties are related.[116–117] Literature have pointed out quercetin, as well as ergosterol (**79**), as anti-inflammatory agents,[113,118,119,120] indicating their possible effect on protecting patients from severe inflammation induced by SARS–CoV–2 infection.[33] Indeed, quercetin (**82**) is the focus of several studies to evaluate its effect on prophylaxis and or treatment for COVID–19, according to WHO ICTRP and ClinicalTrials.gov databases (Fig. 12).

9. Conclusion

Fungi have long been known as sources of metabolites with great diversity, and many them exhibit activity against diverse human emerging and reemerging pathogenic viruses. The "arsenal" of candidate molecules produced by fungi, with a broad spectrum of antiviral activities, encourages continuous efforts to explore the potential of this chemical library in drug discovery programs. The lack of effective drug treatment for SARS-CoV and MERS-CoV, as well as the potential of coronaviruses to cause epidemics, emphasizes the need for novel drugs to treat CoV infections associated with immunization programs. The use of technological approaches to diversify fungal metabolic pathways, automated pharmacological testing, computational molecular design and docking have been guiding further *in vitro* and *in vivo* studies to find suitable drug leads against viruses. In these multidisciplinary scenarios, fungal metabolites are promising sources of compounds that can interfere with different targets of virus life cycles, as shown in some ongoing studies searching for drugs to fight SARS-CoV-2 infection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

JAT proposed the review topic. All authors participated in drafting, critically revised, read and approved the final submitted version. BVRB, LPSP and JAT draft and revised the chemical structures. MNSL drew Figs. 4–6.

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