Starting Anti-COVID-19 Drug Discovery with Natural Products

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ABSTRACT

COVID-19 was characterized as a pandemic regarding its rapid international spread and severity on March 2020. The Coronaviridae family receives this name regarding the organization of the spike glycoprotein located in the envelope, which resembles a stellar corona when observed under a microscope. Coronaviruses undergo frequent mutations in their genome due to errors made by RNA-dependent RNA polymerase (RdRp). The SARS-CoV-2 was characterized by high infectivity and person to person transmission, with an incubation period of up to fourteen days. Potent antiviral activities of several natural products such as alkaloids, chalcones, triterpenoids have been reported but with unconfirmed efficacy or safety in the clinic as well as the complete underlying mechanisms. Also, CQ, HCQ and Ivermectin, remdesivir, lopinavir, ritonavir, favipiravir and pegylated interferon with ribavirin have been tested to develop both an effective therapy and a vaccine to treat COVID-19. This study investigated the antiviral effects of the natural products against SARS-CoV, HCoV-NL63, HCoV-229E and HCoV-OC43. Also, the Lycorine, Emodin, Promazine, Saikosaponins B2, Silvestrol, Cepharanthine, Fangchinoline, Tetrandrine, Caffeic acid, Chlorogenic acid, Gallic acid and Emetine are considered an important hit compounds for prospective anti-SARS-CoV-2 drug discovery.

Keywords: Coronaviruses; Drug Discovery; Natural Products; Transmission.
In December 2019, a new coronavirus was detected in patients with a type of viral pneumonia who, coincidentally, had visited the seafood market in the city of Wuhan, Hubei Province, China (Gorbalenya et al. 2020). In December 2019, the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported by the World Health Organization (WHO). In January 2020, the disease caused by SARS-CoV-2 was called coronavirus disease 19 (COVID-19). The SARS-CoV-2 was characterized by high infectivity and person to person transmission, with an incubation period of up to fourteen days (Cheng et al. 2006). COVID-19 was characterized as a pandemic regarding its rapid international spread and severity (WHO 2020). According to the International Virus Taxonomy Committee (ICTV), SARS-CoV-2 is classified under the order of Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, genus Beta-coronavirus, subgenus Sarbecovirus (de Groot et al. 2020). Coronaviridae is a family of positive-sense RNA viruses, enveloped with nonsegmented RNA genome (Fung & Liu 2019).

The Coronaviridae family receives this name due to the organization of the spike (S) glycoprotein located in the envelope, which resembles a stellar corona when observed under a microscope (Schoeman & Fielding 2019). This Coronaviridae family is subdivided into two subfamilies: Letovirinae and Orthocoronavirinae, comprising 26 subgenera, 46 species. The subfamily Letovirinae has one genus, Letovirinae, and the subfamily Orthocoronavirinae has four genera Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus (WHO 2020). Alphacoronaviruses and Betacoronaviruses infect or cause disease in mammals, including humans (Abolnik 2015), Deltacoronaviruses are related to diseases in birds and pigs (Paim et al. 2019), whereas Gammacoronaviruses are related to diseases in birds, dolphin, and whales (Mihindukulasuriya et al. 2008). Coronaviruses, like other RNA viruses, undergo frequent mutations in their genome due to errors made by RNA-dependent RNA polymerase (RdRp), presenting an average replacement rate of $\sim 3 \times 10^{-4}$ replacements per location per year (Fung & Liu 2019; Pyre et al. 2006; Su et al. 2016). The high frequency of recombination is another important aspect in the genetics of coronaviruses that can have significant effects on the pathogenesis and epidemiology of these viruses (Makino et al. 1986; Su et al. 2016). Besides, the large genome of coronaviruses, in relation to other viruses, with single-stranded RNA allows plasticity in modifying the genome through mutations and recombination, increasing the probability of intra-species and interspecies variability (Su et al. 2016).

The SARS-CoV-2 is the result of viral recombination with the ability to break the biological barrier and escape the animal-animal cycle and infect humans by characterizing a zoonosis that, hypothetically, has the bat as the primary host (Q. Li et al. 2020). However, human to human transmission is what potentiates the epidemic characteristic of these infections (N. Zhu et al. 2020).
such as the epidemics caused by SARS-CoV and the Middle East respiratory syndrome (MERS)-CoV (Zhong et al. 2003). For the SARS-CoV-2 infection process to occur, glycoprotein S needs to be activated to bind to the incoming cell receptor. The initiation and activation process of glycoprotein S is mediated by host cell proteases, with the Transmembrane Protease Serine 2 (TMPRSS2) (Hoffmann et al. 2020) standing out and the angiotensin-converting enzyme receptor 2 (ACE2) is linked to the infection mechanism of some coronaviruses, among them SARS-CoV, responsible for the epidemic peak of this infection that occurred in China in 2002 (W. Li et al. 2003). The SARS-CoV-2+glycoprotein S complex very efficiently uses ACE2 to enter cells, mainly in lung cells, correlating with the rapid spread of SARS-CoV-2 among humans (Walls et al. 2020).

**STRUCTURE AND BIOCHEMISTRY**

Coronaviruses genomic RNA has approximately 30 kb in length, with 14 open reading frames (ORF). The ORF1a and ORF1ab comprise approximately two-thirds of the genome and encodes a complex replicase machinery. Genes for four structural proteins (Spike, Envelope, Membrane, Nucleocapsid) may be found at the 3' end of the virus genome (Romano et al. 2020). An envelope-anchored spike protein mediates coronavirus entry into host cells by first binding to a host receptor and then fusing viral and host membranes (Wan et al. 2020). Two receptors in humans cells have been described as possible routes of virus entrance, the angiotensin-converting enzyme 2 (ACE2) and CD147, both mediated by the spike (S) protein (Luan et al. 2020; K. Wang et al. 2020). The S protein is a multifunctional molecular machine that mediates coronavirus entry into host cells. It first binds to the ACE2 or CD147 receptors on the host cell surface through its S1 subunit and then fuses viral and host membranes through its S2 subunit (Wang et al. 2020). To engage a host cell receptor, the receptor-binding domain (RBD) of S1 undergoes hinge-like conformational movements that transiently hide or expose the determinants of receptor binding. Because of the indispensable function of the S protein, it represents a target for antibody-mediated neutralization, or its receptors may act as candidates for vaccine antigens (Wan et al. 2020; Shang et al. 2020).

The SARS-CoV-2 shares 79.6% of sequence identity to SARS-CoV and is 96% identical at the whole-genome level to BatCoV RaTG13 (a bat coronavirus) (Zhou et al. 2020). The SARS-CoV-2 shares the same receptor that SARS-CoV and RaTG13 (ACE2) in humans (Shang, Ye, et al. 2020; Wan et al. 2020; Li 2005). The crystal structure of the receptor-binding domain (RBD) of SARS-CoV-2 shows a more compact conformation in comparison to the SARS-CoV RBD. Moreover, several residue changes in the SARS-CoV-2 RBD stabilize two virus-binding hotspots at the RBD–ACE2 interface resulting in increased binding affinity (Shang Ye et al. 2020; Wrapp et al. 2020). Five out of six critical
amino acid residues in the spike protein for binding to ACE2 receptors differ between SARS-CoV-2 and SARS-CoV, as the RBD in the spike protein is the most variable part of the coronavirus genome (Zhou et al. 2020; Wrapp et al. 2020). The analysis of the ACE2-binding ridge in SARS-CoV-2 supports the notion that SARS-CoV-2 is associated with RaTG13 or a RaTG13-related bat coronavirus since both viruses contain a similar four-residue motif in that region. These features may have facilitated SARS-CoV-2 to transmit from bats to humans since small differences between the RaTG13 and SARS-CoV-2 spike proteins enhanced ACE2 recognition in the later (Shang Ye et al. 2020).

**CORONAVIRUS GLYCOPROTEINS**

The spike glycoprotein (S) called CoV S protein is the main structural protein in the coronavirus envelope. This protein forms projections about 20 nm in length and is the most polymorphic protein among coronaviruses organized as dimers or trimers. CoV S protein has two well-known functions, which are to fix and activate the fusion of the viral envelope with the host cell membrane, which contributes to the release of the viral genome into the cell (Bosch et al. 2003; Ou et al. 2020). CoV S protein has ~1,200aa of length and is cleaved post-translation into two subunits: amino-terminal S1 and carboxy-terminal S2, with about 500 and 600aa, respectively. The S protein is anchored by the carboxy-terminal portion of the S2 subunit in the viral envelope through a small hydrophobic transmembrane segment, forming the support of the spike, while the S1 subunit is globular and forms the part of the bulb present in the ectodomain of this protein (Binns et al. 1985; Spiga et al. 2003).

The S1 subunit contains the main epitopes targeted for the combination with neutralizing antibodies, which are made up of certain amino acid sequences that confer the serotype specificity to each viral strain (X.-J. Yu et al. 2003; Ou et al. 2020). Another characteristic of the S1 subunit is that it has regions in its sequence that have great variability, regions that are called: hypervariable region 1 (HVR I), delimited by amino acid residues 38 to 69; hypervariable region 2 (HVR II), delimited by amino acid residues 91 to 141; and the hypervariable region 3 (HVR III) comprising amino acid residue 250 to 387. Several studies have demonstrated the importance of these three hypervariable regions in the direct interaction with neutralizing antibodies and, therefore, constitute the serotype-specific determinants presented by the different strains of coronavirus (Takiuchi et al. 2007; Jaimes et al. 2020). Regarding viral protein structures are important tools on immunopathological understanding, the S1 subunit anchored in the ECA 2 receptor in pneumocytes II regulates transmissions between species and from human to human (Geng Li et al. 2020; W. Li et al. 2003).
Angiotensin-converting enzyme 2 (ACE2) is an 805 amino acid metalloprotease that has considerable homology with ACE (Tipnis et al. 2000). ACE2 is a type I membrane protein that acts as a carboxypeptidase and not as a dipeptidyl carboxypeptidase like ACE (Guy et al. 2003). It is expressed in kidney cells, heart, blood vessels, gastrointestinal tract, liver, spleen, brain, placenta, and lungs (Gang Li, Hu & Zhang 2020). ACE2 is highly expressed in the kidneys and the cardiovascular and gastrointestinal systems, being expressed at lower levels in the central nervous system, lymphoid tissue, and lungs (Donoghue et al. 2000; Xudong et al. 2006; Valdés et al. 2006). In lungs, ACE2 is mainly expressed in the pulmonary AT2 alveolar epithelial cells, which are particularly prone to viral infections (Uhal et al. 2011).

ACE2 exhibits biochemical activities different from ACE, converting angiotensin I into angiotensin-(1-9) (Donoghue et al. 2000). Angiotensin-(1-9), whose actions have not yet been well defined, is hydrolyzed by ACE to angiotensin-(1-7). Another action of ACE2 is the hydrolysis of angiotensin II, with the removal of an amino acid, producing, from there, angiotensin-(1-7) (Crackower et al. 2002). The catalytic action of ACE2 is approximately 500x more efficient when the substrate is angiotensin II, compared to its action on angiotensin I. ACE2 is, moreover, 10–600x more potent than prolyl endopeptidase and prolyl-carboxypeptidase, respectively, to generate angiotensin-(1-7) from angiotensin II (Vickers et al. 2002). Several studies have shown that ACE2 expression in human tissues correlates with SARS-CoV and SARS-CoV-2 infection sites, including lung, kidney, and intestine (Sungnak et al. 2020; Zhao et al. 2020), which may be associated with complications in these organs resulting from the SARS-CoV-2 infection.

Similar to SARS-CoV infection, the S1 subunit of the CoV S protein contains a conserved Receptor-Binding Domain (RBD), which recognizes the host cell's angiotensin-converting enzyme 2 (ACE2). The CoV S protein is activated and cleaved by the the membrane-anchored serine transmembrane protease 2 (TMPRSS2), and the typically intracellular cysteine proteases cathepsin B/L, and/or furin, allowing the virus to release fusion peptides and fuse with the membrane. The co-expression of ACE2 and TMPRSS2, cathepsin, and/or furin is a key determinant for SARS-CoV-2 entry into host cells (Hoffmann et al. 2020; Kawase et al. 2012; Coutard et al. 2020). Thus, the virus promotes a decrease in the expression of ACE2, which would be responsible, physiologically, for protective actions of the lung due to its negative regulation on the activity of the renin-angiotensin system that is reported as harmful to the lungs. Thus, the decrease in ACE2 activity affects the exacerbation of atrophic, fibrotic, pro-oxidant, and vasoconstrictor processes in the lung (Zhao et al. 2020).
Coronavirus Proteases

Similar to the SARS-CoV and the MERS-CoV, the SARS-CoV-2 genome encodes nonstructural proteins (such as papain-like protease (nsp3), 3-chymotrypsin-like protease (nsp5), helicase (nsp13), and RNA-dependent RNA polymerase (nsp12)), structural proteins (such as spike glycoprotein) and accessory proteins (Woo et al. 2010; Lai 1990). The four mentioned nonstructural proteins are key enzymes in the viral reproduction, and the spike (S) glycoprotein plays an essential role in the entry of the virus to the host cells. Accordingly, each of these five proteins is recognized as attractive targets to design and develop antiviral agents against SARS and MERS. The genome of SARS encodes two polyproteins, namely *ppla* and *pplb*. These polyproteins are cleaved to different functional proteins by the papain-like protease (*PL* *pro*) and/or the 3-chymotrypsin-like protease (*3CL* *pro*), which is frequently referred to as main protease (*M* *pro*) (Ziebuhr, Snijder & Gorbalenya 2000).

Proteases are enzymes whose catalytic function is to hydrolyze peptide bonds of proteins. Based on the catalytic mechanisms, proteases are classified into nine different groups: metallo, aspartic, cysteine, serine, glutamic, asparagine, threonine, mixed catalytic type, and unknown catalytic type proteases (Rawlings et al. 2018). Coronavirus proteases belong to the cysteine protease class (Lai 1990). Cysteine proteases hydrolyze a peptide bond using the thiol group of a cysteine residue as a nucleophile. Hydrolysis usually involves a catalytic triad consisting of the thiol group of the cysteine, the imidazole ring of a histidine, and a third residue, in most of them, to orientate and activate the imidazole ring (Báez-Santos, St. John & Mesecar 2015). Although the primary function of *PL* *pro* and *3CL* *pro* is to process the viral polyprotein in a coordinated manner, *PL* *pro* has the additional role of stripping ubiquitin and ISG15 from host-cell proteins to aid coronaviruses in their evasion of the host innate immune responses. Therefore, targeting *PL* *pro* with antiviral drugs may have an advantage in not only inhibiting viral replication but also inhibiting the dysregulation of signaling cascades in infected cells that may lead to cell death in the surrounding, uninfected cells (Anand et al. 2003).

SARS-CoV-2 *PL* *pro* X-ray structure – unbound (Ratia et al. 2014) or inhibitor-complexed (Ghosh et al. 2009, 2010; Báez-Santos et al. 2014)- have been investigated by several authors. Báez-Santos et al. grouped the known SARS-CoV-2 *PL* *pro* inhibitors into six different classes, one of them being the inhibitors of natural origin. Among the natural products, *tanshinones*, *diarylheptanoids*, and *geranylated flavonoids* were mentioned (Báez-Santos et al. 2014). Kim et al. found some phenolic components of ethanol extract of the seeds of *Psoralea corylifolia* to show high activity against the SARS-CoV *PL* *pro* with an IC\textsubscript{50} value of 15 µg/ml (D. W. Kim et al. 2014). The structure of the compounds – belonging to the geranylated flavonoids class - are shown in Figure 1. Furthermore, Lee et al.
demonstrated that the MERS-CoV PL$_{pro}$ blocking loop 2 (BL2) structure significantly differs from that of SARS-CoV PL$_{pro}$, where it has been proven to play a crucial role in SARS-CoV PL$_{pro}$ inhibitor binding (H. Lee et al. 2015). The authors tested four SARS-CoV PL$_{pro}$ lead inhibitors against MERS-CoV PL$_{pro}$. None of them were effective against MERS-CoV PL$_{pro}$. Recently, Arya et al. performed an in silico docking studies of several FDA approved drugs and the homology model of the SARS-CoV PL$_{pro}$ enzyme. In this study, sixteen FDA approved drugs, including chloroquine and formoterol, was found to bind the target enzyme with significant affinity and proper geometry, suggesting their potential to be utilized against the virus (Arya et al. 2020).

The indispensable role of coronavirus main protease (Main$_{pro}$) in its essential role in processing the polyproteins that are translated from the viral RNA, makes it a popular target for drug design. Following the first SARS coronavirus outbreak in 2002, a series of inhibitors of Main$_{pro}$ of the SARS-CoV was reported (Bacha et al. 2004; Jain et al. 2004; Wu et al. 2004). These early studies concentrated on (1) testing peptidomimetics, similar to the HIV protease inhibitors ritonavir and lopinavir, and (2) analogs of the natural products which had been reported to show antiviral and antimicrobial effects (L. Wang et al. 2015; Zaman et al. 2017). Wu et al., for example, used a cell-based assay to screen more than 10,000 compounds, all administered at 10 μM, for inhibition of the cytopathic effect of the SARS-CoV virus. The authors found valinomycin, a peptide insecticide, to be the most effective of the screened compounds with an EC$_{50}$ of 0.85 μM. Beta-aescin and reserpine were also found active. Compounds that are structurally similar to reserpine and beta-aescin were also tested, resulting in 10 new anti-SARS active compounds (Wu et al. 2004). Based on the high similarity of M$_{pro}$s in each CoV group, some wide-spectrum Michael acceptor peptidomimetic inhibitors (I2, N1, and N3) targeting SARS-CoV Main$_{pro}$ enzymes (Figure 2) (Yang et al. 2005).

Yang and coworkers was first publishing the X-ray analysis of the SARS-CoV Main$_{pro}$ holoenzyme and its complex with its inhibitor, chloromethyl ketone (CMK) (Yang et al. 2003). In contrast to common serine proteases, which have a Ser–His–Asp catalytic triad, SARS M$_{pro}$ has a Cys–His catalytic dyad (Cys-145 and His-41), which is similar to the TGEV M$_{pro}$ (Cys-144 and His-41) and the HCoV 229E M$_{pro}$ (Cys-144 and His-41) (Anand et al. 2003). Jin et al. reported on the crystal structure of the SARS-CoV-2 M$_{pro}$-N3 complex, demonstrating a similar binding mode in the SARS CoV M$_{pro}$-N1 and the SARS CoV-2 M$_{pro}$-N3 complexes. The authors used a FRET assay to screen about 10,000 compounds, consisting of approved drugs, clinical-trial drug candidates, and natural products. The primary hits were seven compounds, including disulfiram, carmofur, and ebselen (Jin et al. 2020).
Some docking studies to find homoioflavanones and their thio analogs as inhibitors of the mutant coronavirus main protease enzyme SARC-CoV-2 Mpro were performed (Sepay et al. 2020). Based on the binding properties, they predicted the homoiothioflavone 7 (Figure 3) as a prosperous candidate for further investigations (Sepay et al. 2020). Furthermore, the structure-based virtual screening and molecular dynamic simulation indicated natural compounds with strong binding affinity and the ability to inhibit the SARS-CoV-2 Mainpro enzyme. The authors had a library of 1,048 natural compounds isolated from edible African plants. Rhamnetin (8) and ellagic acid (9) (Figure 3) showed a better therapeutic prediction than compound N3 used as standard (Azhagiya Singam et al. 2020).

**HOST CELL PROTEASES**

As for the natural product-based antiviral strategies, each step of the viral life cycle could be taken into consideration. The virus replication cycle can be divided into six structurally and biochemically different stages: (1) attachment of the virus to receptors on the host cell surface; (2) entry of the virus through the host cell membrane; (3) uncoating of viral nucleic acid; (4) replication, involving (a) synthesis of early regulatory proteins, (b) synthesis of new viral RNA or DNA; and (c) synthesis of late, structural proteins; (5) assembly (maturation) of viral particles; and (6) release from the host cell (Lindenbach 2013). Successful drug development can be seen considering each approach.

Coronaviruses can enter cells via fusion either directly at the cell surface or can be internalized through the endosomal compartment. The attachment of coronaviruses to the surface of the host cells is based on the formation of a coronavirus S protein and the ACE2 receptor of the host cell. The interactions, which result in the entry of the virus, also require activation of the S protein by the cellular proteinases (Kawase et al. 2012; Coutard et al. 2020). ACE2 is a metalloproteinase of which physiological function is hydrolytic cleavage is the protein angiotensin 2 (Tipnis et al. 2000; Heurich et al. 2014). It most often resides on the surface of epithelial cells that cover the surface of internal body cavities, such as the walls of the heart, intestines, and alveoli. However, not only angiotensin-2 but also the spike proteins of the SARS-CoV viruses can bind to the ACE2 receptors, making this receptor the key to viral cell infection. Inhibition of ACE2 enzymic activity by natural or synthetic inhibitors is not without risks. The main function of ACE2 is to inactivate angiotensin-2, the octapeptide with hypertensive activity. If we reduce the activity of ACE2, angiotensin-2 will build-up, which raises blood pressure and can trigger dangerous inflammatory processes (Heurich et al. 2014).

By this time, inhibition of the transmembrane protease serine 2 resulted in several prosperous results in blocking the attachment of coronaviruses in the surface of the host cells. TMPRSS2 facilitates human coronaviruses SARS-CoV and SARS-CoV-2 infections via two independent mechanisms, (a)
proteolytic cleavage of ACE2 receptor, which promotes viral uptake (Heurich et al. 2014), and cleavage of coronavirus spike glycoproteins which activates the glycoprotein for host cell entry (Hoffmann et al. 2020). Inhibition of the latter activity of TMPRSS2 by camostat mesylate (NI-03) (Figure 4), proved to be a clinically approved approach to block entry of the SARS-CoV 2 virus into mammalian cells and might constitute a treatment option (Hoffmann et al. 2020).

**Coronavirus RNA-Dependent RNA-Polymerase**

Molecular biological studies indicated that all structural proteins (including the spike (S), envelope (E), membrane (M), hemagglutinin-esterase (HE), and nucleocapsid (N)) show considerable structural variations among the different CoVs (Woo et al. 2005; Shen et al. 2019; Marra et al. 2003; Rota et al. 2003). On the contrary, protein structural analyses suggest that key binding pockets of the RNA-dependent RNA-polymerase (RdRs) enzymes are likely to be conserved across the three CoV viruses. Therefore, it is reasonable to use data from agents already assessed for activity against SARS/MERS CoVs to extrapolate to SARS-CoV-2. Accordingly, the RNA-dependent RNA-polymerase (similar to RNA helicase, and the main proteinase (Mpro,20) constitute attractive nonstructural protein targets for the development of wide-spectrum anti-coronavirus drugs.

Coronaviruses are single-stranded, positive-sense, nonsegmented RNA viruses (Class IV) according to the Baltimore scheme (Baltimore 1971). They can use their genome both as genomic and mRNA. One of the viral genes expressed yields an RNA-dependent RNA-polymerase (or RNA replicase), which creates minus-strand RNA from the plus-strand genome. The minus-strand RNA can be used as a template for more plus-strand RNA, which can be used as mRNA or as genomes for the newly forming viruses. Present-day inhibitors of such activities of RNA-dependent RNA-polymerases are nucleoside analogs. The approved nucleoside analogs favipiravir and ribavirin have been tested against SARS and MERS and shown to have antiviral effects *in vitro*. Clinical results have suggested that favipiravir affected patients with COVID-19, and it has been added to the list of potential therapies there (Cai et al. 2020). Ribavirin is a guanosine analog with *in vitro* activity against a large number of highly lethal emerging viruses (Figure 5). Ribavirin inhibits RNA synthesis by viral RdRp as well as inhibiting mRNA capping. Studies demonstrated, however, that while SARS-CoV, MERS-CoV, and HCoV-OC43 were sensitive to ribavirin *in vitro*, doses that significantly inhibited CoV replication exceeded ribavirin concentrations attainable by typical human regimens (Falzarano et al. 2013).

Remdesivir (Figure 5) was initially studied as a possible treatment for Ebola but was not particularly effective in comparison to other agents investigated in these outbreaks. It is effective in *in vitro* tests and animal models of SARS and MERS. Additional studies demonstrated that remdesivir
decreased viral titers and viral RNA in *in vitr*o models of both SARS-CoV and MERS-CoV infection of HAEs (Sheahan et al. 2017).

Furthermore, Yin *et al.* (Yin et al. 2020) reported the structure of the SARS-CoV-2 RdRp either in the apo form or in complex with a 50-base template-primer RNA and remdesivir (GS-5734). The complex structure revealed that the partial double-stranded RNA template was inserted into the central channel of the RdRp, where remdesivir is incorporated into the first replicated base pair and terminates the chain elongation (Yin et al. 2020). The results can provide a rational basis to design even more potent SARS-CoV RdRs inhibitors. One of the prosperous candidates is galidesivir (BCX4430). It has broad-spectrum antiviral effectiveness against a range of RNA virus families, including coronaviruses (SARS and MERS) (Zumla et al. 2016).

**CORONAVIRUS RNA HELICASE**

To convert a closed double-stranded DNA or RNA helix into two open single strands, so that other protein machinery can manipulate the polynucleotides, the cells require helicases. Helicases are small molecular motors that use energy derived from ATP hydrolysis. They are classified into six superfamilies SF1-SF6 and participate in almost every aspect of nucleic acid metabolism. DNA helicases play key roles in DNA replication, recombination, and repair. Cells need RNA helicases for transcription, translation, and RNA splicing. Earlier, several potent antiviral drugs were discovered that inhibit an essential herpes simplex virus (HSV) helicase complex, and this discovery inspired many others to study helicases as drug targets (Guang Xi 2007; Kwong, Rao & Jeang 2005; Shadrick et al. 2012).

The majority of helicases prefer only one type of nucleic acid (*i.e.*, either RNA or DNA) as an unwinding substrate (Frick 2003). Regardless of their functional diversity, helicases all contain core domains that hydrolyze nucleoside triphosphates. The enzymatic core is formed either by the tandem RecA-like domains within the same polypeptide chain (SF1-SF2 superfamilies) or between subunits of the functional oligomer of the helicase (SF3-SF6 superfamilies) (Singleton, Dillingham & Wigley 2007). Sequence conservation analysis indicates that SARS CoV helicase belongs to the SF1 superfamily, including Rep, UvrD, PcrA, RecD, Pif1, Dda, Upf1-like helicases, and many positive ss RNA virus helicases (Gorbalenya & Koonin 1989).

Hao and co-workers first reported on the X-ray structure of MERS CoV helicase (Hao et al. 2017). The authors found that MERS-CoV helicase has multiple domains, including an N-terminal Cys/His rich domain (CH) with three zinc atoms, a beta-barrel domain, and a C-terminal SF1 helicase
core with two RecA-like subdomains. It was found that while the domain organization of helicases (nsp13 proteins) is conserved throughout nidoviruses, the individual domains of them are closely related to the equivalent eukaryotic domains of Upf1 helicases. Earlier, Yu and co-workers conducted in vitro biochemical experiments to find out which natural compounds might suppress either (1) the DNA unwinding activity or (2) the ATPase activity of the SARS CoV helicase. The authors demonstrated that selected naturally-occurring flavonoids, including myricetin (12) and scultellarein (13) (Figure 6), might serve as SARS-CoV chemical inhibitors (Yu et al. 2012).

Tanner and coworkers (Tanner et al. 2005) have found that bananin (14) (Figure 7) and its three derivatives function as noncompetitive SARS-CoV helicase inhibitors at a site different from the ATP and nucleic acid binding site, causing inhibition probably through an allosteric mechanism. Lee et al. discovered a novel chemical compound, \((E)-3-(\text{furan-2-yl})-N-(4-\text{sulfamoylphenyl})\alpha\text{rylamide} (15)\) (Figure 7) that suppresses the enzymatic activities of SARS coronavirus helicase. The authors performed ATP hydrolysis and double-stranded DNA unwinding inhibitory assays and found IC\(_{50}\) values of the \(\mu\text{M}\) range (Lee et al. 2017). Pillaiyar et al. recently published a comprehensive survey on the chemical structures that have proved to be effective against SARS and MERS viruses according to the different potential targets. In their review, they did not mention any helicase inhibitors approved for antiviral therapy. The most promising candidate has been a synthetic 1,2,4-triazole derivative \(16\) (SSYA10-001) that inhibited the viral NTPase/helicase of both SARS-and MERS-CoVs (Pillaiyar, Meenakshisundaram & Manickam 2020). Based on the results, compound \(16\) (Figure 7) could serve as a potential lead for the development of effective broad-spectrum anti-coronavirus drugs.

**NATURAL PRODUCTS**

For centuries, the natural products and their derivatives have been recognized as inexhaustible sources of therapeutic agents (Rodrígues et al. 2016; Zhi et al. 2019; Molinari 2009). The molecular structures and supramolecular arrangements of natural products have continued to inspire the development new drug entity (Molinari 2009; Viegas, Da Silva Bolzani & Barreiro 2006). The treatment of viral infections has witnessed the use of extracts, formulations, active principles of plant origin (S. Hu et al. 2019). For instance, plant species rich in quinoline alkaloids belonging to the Rutaceae family (Almeida coerula, Araliopsis tabouensis, Bornellia koniamiensis, Brombya sp. nov., Esenbeckia pentaphylla, Evodia fargesii, Haplophyllum sieversii, Melicope bonwickii, Melicope semecarpifolia, Philotheca deserti var. deserti, Pilocarpus grandiflorus, Ruta chalepensis, Spathelia excelsa, Spiranthera odoratissima, Toddalia aculeata , Zanthoxylum ailanthoides, Zanthoxylum heitzii) posesses antiviral property (da Silva et al. 2013).
Potent antiviral activities of several alkaloids, chalcones, triterpenoids have been reported (Islam et al. 2020). In the previous study, alkaloids that were isolated from the root bark of *Z. ailanthoides* inhibited viral replication in H9 lymphocyte cells without significant impact on the growth of uninfected H9 cells (Chouhan et al. 2014). In order to ensure therapeutic success of plant extract or isolates in the treatment of COVID-19, the screening for antiviral activities needs to take account of the coronavirus machinery for replication and pathophysiological mechanistic. The extracts with phytocconstituents that exhibit activities against coronavirus as shown in Table 1.

Current challenges with the therapeutic application of extracts and active principles of plant origin in the treatment of coronavirus infection include unconfirmed efficacy or safety in the clinic as well as the complete underlying mechanisms (Li et al. 2005). The emergence and spread of resistant strains, as well as the high rates of mutations in microorganisms, reduced the effectiveness of antiviral drugs (Gerrish and García-Lerma 2003). Altogether, there are needs to continually develop new antiviral therapies with wide or narrow spectrum in order to solve drug resistance problems or find new molecules and novel mechanisms of action in the treatment of viral diseases.

**Synthetic Derivatives**

*Chloroquine* (17) and hydroxychloroquine (18) are synthetic derivatives of quinine (19), a quinolinic alkaloid that was isolated from the bark of a *Cinchona* tree by pharmacists Pierre Joseph Pelletier and Joseph Caventou, around 1920 (Figure 8). For decades the salts of chlorate or phosphate of chloroquine (CQ) and hydroxychloroquine (HCQ), both 4-amoquinolines, have been used to treat malaria, an infectious disease caused by species of *Plasmodium spp*. CQ has been known since 1934 and HCQ was synthesized in 1946 by introducing an N-hydroxy-ethyl side chain in place of CQ's N-diethyl group (Monteiro et al. 2020). HCQ proved to be (~40%) less toxic (McChesney 1983) during its prolonged use (for months or even years), allowing the application of higher doses than QC (Marmor et al. 2016) (25 mg/kg for 3 days). Therefore, HCQ is recommended for the treatment of autoimmune diseases, such as: lupus erythematosus systemic and rheumatoid arthritis (Lim et al. 2009).

Although they are generally safe substances when used to treat malaria and autoimmune diseases, the safety, efficacy, and benefit of these drugs in the treatment of COVID-19 (SARS-CoV-2) have been analyzed by several research groups around the world. The CQ acts on lung cell receptors and interferes with the glycosylation of the angiotensin receptor by converting enzyme 2 (ACE2), thus, impairing the entry of viruses into cells, since SARS-CoV-2 invades lung cells by endocytosis through the ACE2 receptor (W. Li et al. 2003). According to Hu and collaborators, QC induces the suppression of phosphatidylinositol binding clathrin assembly protein (PICALM), preventing, in this way,
endocytosis (Hu, Frieman & Wolfram 2020). The alkalinization of lung cells promoted by 4-aminophenylalanine also hinders viral replication, as it impairs the function of the endosome, inhibits the fusion of autophagosome-lysosomes, in addition to inactivating enzymes that are essential for viral replication (Savarino et al. 2003).

The *in vitro* antiviral activity of QC (Inglof 1969) was first verified in the late 1960s, when the ability of this drug to inhibit the synthesis of encephalomyocarditis virus was demonstrated. Currently, there are in vitro studies that have suggested an anti-SARS-CoV activity (Keyaerts et al. 2004) of the QC and HCQ salts. Liu et al. (Yao et al. 2020) recommend using HCQ sulfate (400 mg given twice a day, followed by 200 mg twice a day for another 4 days) to treat patients infected with SARS-CoV-2. Considering the usage history of these drugs, their safety and their low cost, these authors recommended that clinical studies be carried out to evaluate the effects of the drug in patients infected with COVID-19.

The effectiveness of QC phosphate in humans for the treatment of severe acute respiratory syndrome caused by the coronavirus was initially reported by Chinese scientists Gao and collaborators. However, this publication, available in Preprints repositories, does not present scientific data to support such findings and, therefore, cannot be taken as conclusive (Gao, Tian & Yang 2020). Despite this, the Republic of China, through the National Health Commission, included QC in its sixth edition of the Guidelines for the prevention, diagnosis, and treatment of pneumonia induced by the new coronavirus (Dong, Hu & Gao 2020). Chinese guidelines recommend using doses of 300 mg to 500 mg (twice daily) of QC phosphate over a period of 10 days. A preliminary clinical study conducted in the south of France verified the efficiency of HCQ sulfate 600 mg/d (200 mg, three times per day, during ten days) in reducing viral load in patients diagnosed with COVID-19. Gautret and collaborators reported that the elimination of the virus was more efficient when azithromycin was associated with HCQ (Gautret et al. 2020).

In Brazil, a randomized, double-blind trial, conducted by Borba and colleagues, with 81 hospitalized patients due to SARS-CoV-2 infection, showed that the highest dose of chloroquine (600mg CQ twice daily for 10 days) should not be recommended for critically ill patients with COVID-19 for safety reasons due to prolonged QTc interval and increased lethality (Borba et al. 2020). The study also found that the potential risk to safety (safety hazards) of patients increases, especially when QC is administered in combination with azithromycin and oseltamivir. Despite the effectiveness of QC or HCQ in laboratory studies, reports in the literature (Wang et al. 2015) describe the difficulty and even the disappointment when transposing it to a clinical scale. The complex pharmacokinetics of 4-
aminoquinolines justify these differences between the results of laboratory and clinical trials (Lim et al. 2009; Smit et al. 2020). 4-aminoquinolines demonstrate high basicity, high half-life (around 40-45 days), lead to accumulation in lysosomes, in addition to having a high volume of distribution and long renal clearance.

The main adverse effects related to the use of CQ and HCQ are related to retinopathy (Saurabh et al. 2013) which can occur even after discontinuation of treatment. Another reported adverse effect is cardiovascular toxicity, due to its electrical instability, characterized by the prolongation of the QT interval, that is, the time spent for ventricular depolarization and repolarization. This mechanism is related to the blocking of the hERG potassium channel, which prolongs ventricular repolarization and the duration of action potentials, triggering ventricular arrhythmias. Studies by Shi et al. and Guo et al. demonstrate that patients who suffer from cardiovascular diseases and are infected with SARS-CoV are more likely to develop ventricular arrhythmias (Shi et al. 2020; Guo et al. 2020).

Therefore, given the lack of sufficient scientific evidence (Geleris et al. 2020) to prove the efficacy and safety of CQ and HCQ to treat patients infected with coronavirus (COVID-19), the World Health Organization has temporarily suspended clinical trials with these drugs. Despite this, countries like China and Brazil (Rosenberg et al. 2020; Mahévas et al. 2020; Tang et al. 2020) maintain guidelines and protocols that suggest they authorize the use of these drugs to treat patients with SARS-CoV-2. Another derivative synthesized, obtained in 1975, and that comes from natural products, is ivermectin. It derives from avermectin, a product that comes from the fermentation of the actinomycete Streptomyces avermitilis, through catalytic hydrogenation of a single double bond. Ivermectin (anti-parasitic) has greater potency and less toxicity than avermectin, which is used for its insecticidal and anthelmintic action. Ivermectin inhibited SARS-CoV-2 in laboratory cultures of infected cells (Vero-hSLAM cells) (Caly et al. 2020).

Ivermectin probably interacts with transmembrane transporters (Impae Impb1) thus preventing the virus from entering the cell nucleus. Consequently, viral multiplication would be inhibited and this would result in decreased infection, as there would be a reduced inhibition of antiviral responses, leading to a more efficient antiviral response. However, the results presented in monkey kidney cell cultures were relatively high (IC$_{50}$ = 2.5 µm), requiring extremely high doses for therapeutic effects in humans and, consequently, already proving not to be an effective drug for the treatment of Covid-19 (Momekov & Momekova 2020). In addition, previous experiments in cell cultures have shown to be effective in treating Dengue virus infection, but have failed in animal
models. Finally, on April 10, 2020, the FDA’s Center for Veterinary Medicine (FDA Letter to Stakeholders: Do Not Use Ivermectin Intended for Animals as Treatment for COVID-19 in Humans FDA n.d.) advised not to use ivermectin that is intended for animals as a treatment for Covid-19 in humans. In addition to CQ, HCQ and Ivermectin, other drugs (not derived from natural products) are being considered, such as: remdesivir, lopinavir, ritonavir, favipiravir and pegylated interferon with ribavirin (Ahn et al. 2020) are also being tested in a constant and global effort by research laboratories and institutions to develop both an effective therapy and a vaccine to treat emerging infectious contagious diseases, such as COVID-19, which is currently unsolved.

**ARTIFICIAL INTELLIGENCE DRIVEN DRUG DISCOVERY**

Although there are already several drugs being assessed clinically for SARS-CoV-2 (Beigel et al. 2020; García et al. 2020; Irvani et al. 2020; Irie et al. 2020), the continuous efforts to discover new drug candidates more effective, safe, and inexpensive remain necessary. However, the accelerated discovery of new anti-COVID-19 drugs represents a major challenge, since this demand is incompatible with current drug discovery pipelines, which require long cycle times of research, and present limited success in clinical trials (Nosengo 2016). To make this process faster and efficient, academies, startups and big pharmaceutical companies are exploring the potential of artificial intelligence (AI) systems to help streamline their research and development (R&D) process (Ekins et al. 2019).

With the recent advances in computer technology, solid progress in AI-drive drug discovery field has been made by using machine learning (ML) techniques. These techniques enable the development of mathematical models from various data types. Once having learned from the data, the ML model can be used to make predictions or decisions without being explicitly programmed to do so. The possibilities of ML seem virtually unlimited, with applications ranging from automation of whole organism assays (Singh, Carpenter & Genovesio 2014), lead identification and optimization (Neves et al. 2018; Chen et al. 2018), early accessing of pharmacokinetics and toxicological (ADME/Tox) profile of compounds (Goh, Hodas & Vishnu 2017), formulation design (Alves et al. 2019), as well as clinical trial recruiting, design and optimization (Gayvert, Madhukar & Elemento 2016). Applying ML to discovery NP with potential anti-SARS-CoV-2 activity is a sequential process that involves the use of algorithms to learn from datasets of compounds with bioactivity data (Figure 9) (Lavecchia 2015).

Initially, chemical and biological data are collected from bioassay databases and the literature and curated using standardized protocols (Fourches et al. 2010; Alves et al. 2016). In this sense, thousands of compounds with experimental ADME/Tox properties, and bioactivity data for SARS-
CoV-2 are publicly available on databases such as ChEMBL (Gaulton et al. 2012) and PubChem Bioassay (Y. Wang et al. 2012). Then, molecular descriptors (i.e., final result of a mathematical procedure which transforms chemical structure into a useful number) are calculated on different levels of representation (1D to nD) of chemical structures (Chuang, Gunsalus & Keiser 2020). In the learning phase, a ML technique is applied to discover an empirical function that can achieve an optimal mapping between the molecular descriptors and experimental property. To date, Support Vector Machine (SVM) (Vapnik 2000), Random Forest (RF) (Breiman 2001), and Deep Neural Networks (Lusci, Pollastri & Baldi 2013) are the most popular and effective ML methods for modeling quantitative structure-activity relationships. At the end of this step, the ML model must be subjected to rigorous statistical validation to determine its predictive power (Cherkasov et al. 2014). Once presenting a satisfactory statistical performance, ML models may be applied as filters in virtual screening (VS) of libraries of natural and semisynthetic compounds (i.e., 103 to 104 chemical structures) (Cherkasov et al. 2014).

In principle, VS is often compared to a funnel, where a large number of natural products in chemical libraries (i.e., 103 to 105 compounds) are reduced to a smaller number of virtual hits that will be tested experimentally (i.e., 101 to 102 compounds) (Tanrikulu, Krüger & Proschak 2013; Kar & Roy 2013). Although in principle, ML models are the most useful tools for the identification of bioactive compounds, complementary computational methods can be incorporated into VS as a multi-step filtering process, including: (i) sets of empirical rules (e.g., Veber (Veber et al. 2002) and Lipinski’s (Lipinski et al. 1997) rules, pan-assay interference rules and models (Baell & Holloway 2010; Jasial et al. 2018)); (ii) chemical similarity cutoffs; (iii) ADME/Tox filters (Braga et al. 2015); (iv) and molecular docking (Kitchen et al. 2004). In this sense, the integration of different methods increases reliability in predictions and the hit rate in VS.

Generally, typical hit rates from experimental VS typically range between 1% and 40%, while the hit rates of experimental random screening approaches range between 0.01% and 0.1% (T. Zhu et al. 2013). Despite the high hit rate, the expectation that ML-based VS can completely replace experimental assays is overoptimistic. So, in vitro experimental validation of computational hits should be performed as the most important step of the study. After experimental validation of virtual hits, ML models can still play a key role in the hit-to-lead and lead optimization design of semisynthetic compound series. In this sense, models can be used as a multi-parameter optimization decision system to find the compounds with adequate balance between potency, selectivity and ADME/Tox properties (Neves et al. 2018).
CONCLUSION

This study investigated the antiviral effects of the natural products against SARS-CoV, HCoV-NL63, HCoV-229E and HCoV-OC43. Also, the Lycorine, Emodin, Promazine, Saikosaponins B2, Silvestrol, Cepharanthine, Fangchinoline, Tetrandrine, Caffeic acid, Chlorogenic acid, Gallic acid and Emetine are considered an important hit compounds for prospective anti-SARS-CoV-2 drug discovery. Further hit-to-lead analyses are required to transform these potential inhibitors into clinical drugs. We anticipate that the insights gained in the present work may prove valuable for exploring novel natural products as anti-COVID-19 therapeutic agents in the future.

REFERENCES

Abolnik C 2015. Genomic and single nucleotide polymorphism analysis of infectious bronchitis coronavirus. Infection, Genetics and Evolution, 32(6): 416–424.

Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, Kim BT, Kim SJ 2020. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 COVID-19). Journal of Microbiology and Biotechnology, 30(3): 313–324.

Alehaideb Z, Kimberly CC, Mei CY, Francis CPL 2019. Predicting the Content of Anthraquinone Bioactive in Rhei Rhizome (Rheum Officinale Baill) with the Concentration Addition Model. Saudi Pharmaceutical Journal, 27(1): 25–32.

Alves VM, Capuzzi SJ, Muratov EN, Braga RC, Thornton TE, Fourches D, Strickland J, Kleinstreuer N, Andrade CH, Tropsha A 2016. QSAR models of human data can enrich or replace LLNA testing for human skin sensitization. Green Chemistry 18(24): 6501–6515.

Alves VM, Hwang D, Muratov E, Sokolsky-Papkov M, Varlamova E, Vinod N, Lim C, Andrade CH, Tropsha A, Kabanov A 2019. Cheminformatics-driven discovery of polymeric micelle formulations for poorly soluble drugs. Science Advances, 5(6): eaav9784.

Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R 2003. Coronavirus main proteinase 3CLpro. Structure): Basis for design of anti-SARS drugs. Science, 300(5626): 1763–1767.

Arya R, Das A, Prashar V, Kumar M 2020. Potential inhibitors against papain-like protease of novel coronavirus SARS-CoV-2. from FDA approved drugs. Chemrxiv, 1–8.

Azhagiya Singam ER, Michele L merrill, Kathleen A. D, Martyn T. S 2020. Structure-Based Virtual Screening of a Natural Product Database to Identify Several Possible SARS-CoV-2 Main Protease Inhibitors Chemrxiv, 1-5.

Bacha U, Barrila J, Velazquez-Campoy A, Leavitt SA, Freire E 2004. Identification of Novel Inhibitors of the SARS Coronavirus Main Protease 3CLpro. Biochemistry, 43(17): 4906–4912.

Baell JB, Holloway G a. 2010. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. Journal of Medicinal Chemistry, 53(7): 2719–2740.
Báez-Santos YM, St. John SE, Mesecar AD 2015. The SARS-coronavirus papain-like protease): Structure, function and inhibition by designed antiviral compounds. *Antiviral Research*, 115): 21–38. https://doi.org/10.1016/j.antiviral.2014.12.015

Báez-Santos YM, Barraza SJ, Wilson MW, Agius MP, Mielech AM, Davis NM, Baker SC, Larsen SD, Mesecar AD 2014. X-ray structural and biological evaluation of a series of potent and highly selective inhibitors of human coronavirus papain-like proteases. *Journal of Medicinal Chemistry*, 57(6): 2393–2412.

Baltimore D 1971. Expression of animal virus genomes. *Bacteriological reviews*, 35(3): 235–241.

Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, Hohmann E, Chu HY, Luetkemeyer A, Kline S, Lopez de Castilla D, Finberg RW, Dierberg K, Tapson V, Hsieh L, Patterson TF, Paredes R, Sweeney DA, Short WR, Touloumi G, Lye DC, Ohmagari N, Oh M, Ruiz-Palacios GM, Benfield T, Fäktenerheuer G, Kortepeter MG, Atmar RL, Creech CB, Lundgren J, Babiker AG, Pett S, Neaton JD, Burgess TH, Bonnett T, Green M, Makowski M, Osinusi A, Nayak S, Lane HC 2020. Remdesivir for the Treatment of Covid-19 — Preliminary Report. *New England Journal of Medicine*, NEJMoa2007764.

Binns MM, Boursnell MEG, Cavanagh D, Pappin DJ, Brown TD 1985. Cloning and sequencing of the gene encoding the spike protein of the coronavirus IBV. *Journal of General Virology*, 66(4): 719–726.

Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, Mourão MPG, Brito-Sousa JD, Baia-da-Silva D, Guerra MVF, Hajjar LA, Pinto RC, Balieiro AAS, Pacheco AGF, Santos JDO, Naveca FG, Xavier MS, Siqueira AM, Schwarzbold A, Croda J, Nogueira ML, Romero GAS, Bassat Q, Fontes CJ, Albuquerque BC, Daniel-Ribeiro CT, Monteiro WM, Lacerda MVG 2020. Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients Hospitalized With Severe Acute Respiratory Syndrome Coronavirus 2 SARS-CoV-2. Infection): A Randomized Clinical Trial. *JAMA network open*, 3(4): e208857.

Bosch BJ, van der Zee R, de Haan CAM, Rottier PJM 2003. The Coronavirus Spike Protein Is a Class I Virus Fusion Protein): Structural and Functional Characterization of the Fusion Core Complex. *Journal of Virology*, 77(16): 8801–8811.

Braga RC, Alves VM, Silva MFB, Muratov E, Fourches D, Lião LM, Tropsha A, Andrade CH 2015. Pred-hERG): A Novel web-Accessible Computational Tool for Predicting Cardiac Toxicity. *Molecular Informatics*, 34(10): 698–701.

Breiman L 2001. Random forests. *Machine Learning*, 45(1): 5–32.

Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, Liao X, Gu Y, Cai Q, Yang Y, Shen C, Li X, Peng L, Huang D, Zhang J, Zhang S, Wang F, Liu J, Chen L, Chen S, Wang Z, Zhang Z, Cao R, Zhong W, Liu Y, Liu I. 2020. Experimental Treatment with Favipiravir for COVID-19): An Open-Label Control Study. *Engineering*, 1-8.

Cai Y, Druce JD, Catton MG, Jans DA, Wagstaff KM 2020. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Research*, 178(6): 104787.

Chen H, Engkvist O, Wang Y, Olivecrona M, Blaschke T 2018. The rise of deep learning in drug discovery. *Drug Discovery Today*, 23(6): 1241–1250.
Cheng PW, Ng LT, Chiang LC, Lin CC 2006. Antiviral effects of saikosaponins on human coronavirus 229E in vitro. *Clinical and Experimental Pharmacology and Physiology*, 33(7): 612–616.

Cherkasov A, Muratov EN, Fourches D, Varnek A, Baskin II, Cronin M, Dearden J, Gramatica P, Martin YC, Todeschini R, Consonni V, Kuz’min VE, Cramer R, Benigni R, Yang C, Rathman J, Terfloth L, Gasteiger J, Richard A, Tropscha A 2014. QSAR modeling: where have you been? Where are you going to? *Journal of Medicinal Chemistry*, 57(12): 4977–5010.

Chor, S. Y., A. Y. Hui, K. F. To, K. K. Chan, Y. Y. Go, H. L.Y. Chan, W. K. Leung, and J. J.Y. Sung. 2005. Anti-Proliferative and pro-Apoptotic Effects of Herbal Medicine on Hepatic Stellate Cell. *Journal of Ethnopharmacology*, 100(1–2): 180–86.

Chouhan G, Islamuddin M, Sahal D, Afrin F 2014. Exploring the role of medicinal plant-based immunomodulators for effective therapy of leishmaniasis. *Frontiers in Immunology*, 5(193).

Chuang K V., Gunsalus LM, Keiser MJ 2020. Learning Molecular Representations for Medicinal Chemistry. *Journal of Medicinal Chemistry*, 63(16): 8705-8722.

Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E 2020. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Research*, 176(4): 104742.

Crackower MA, Sarao R, Oliveira-dos-Santos AJ, Da Costa J, Zhang L 2002. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*, 417(6891): 822–828.

Dong L, Hu S, Gao J 2020. Discovering drugs to treat coronavirus disease 2019 COVID-19). *Drug Discoveries & Therapeutics*, 14(1): 58–60.

Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S 2000. A novel angiotensin-converting enzyme-related carboxypeptidase ACE2. converts angiotensin I to angiotensin 1–9. *Circulation research*, 87(5).

Ekins S, Puhl AC, Zorn KM, Lane TR, Russo DP, Klein JJ, Hickey AJ, Clark AM 2019. Exploiting machine learning for end-to-end drug discovery and development. *Nature Materials*, 18(5): 435–441.

Falzarano D, De Wit E, Martella C, Callison J, Munster VJ, Feldmann H 2013. Inhibition of novel β coronavirus replication by a combination of interferon-α2b and ribavirin. *Scientific Reports*, 3(4):1-8.

Fourches D, Muratov E, Tropscha A, Fourchers D, Muratov E, Tropscha A 2010. Trust, but verify): on the importance of chemical structure curation in cheminformatics and QSAR modeling research. *Journal of Chemical Information and Modeling*, 50(4): 1189–204. https://doi.org/10.1021/ci100176x

Frick DN 2003. Helicases as antiviral drug targets. *Drug News and Perspectives*, 16(1): 355–362.

Fung TS, Liu DX 2019. Human Coronavirus): Host-Pathogen Interaction. *Annual Review of Microbiology*, 73(1): 529–557.

Gao J, Tian Z, Yang X 2020. Breakthrough): Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *BioScience Trends*, 14(1):72-73.
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Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Scaliant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

García IG, Rodríguez-Rubio M, Mariblanca AR, de Soto LM, García LD, Villatoro JM, Parada JQ, Meseguer ES, Rosales MJ, González J, Arribas JR, Carcas AJ, de la Oliva P, Borobia AM 2020. A randomized multicenter clinical trial to evaluate the efficacy of melatonin in the prophylaxis of SARS-CoV-2 infection in high-risk contacts (MeCOVID Trial)): A structured summary of a study protocol for a randomised controlled trial. *Trials*, 21(1): 466.

Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B, Overington JP 2012. ChEMBL): a large-scale bioactivity database for drug discovery. *Nucleic Acids Research*, 40: D1100–D1107.

Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont HT, Honoré S, Colson P, Chabrière E, La Scola B, Rolain J-M, Brouqui P, Raoult D 2020. Hydroxychloroquine and azithromycin as a treatment of COVID-19): results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents*, 56(1): 105949.

Gayvert KM, Madhukar NS, Elemento O 2016. A Data-Driven Approach to Predicting Successes and Failures of Clinical Trials. *Cell Chemical Biology*, 23(10): 1294–1301.

Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, Labella A, Manson DK, Kubin C, Barr RG, Sobieszczyk ME, Schluger NW 2020. Observational Study of Hydroxychloroquine in Hospitalized Patients with Covid-19. *New England Journal of Medicine*, 382(25): 2411-2418.

Gerrish PJ, García-Lerma JG 2003. Mutation rate and the efficacy of antimicrobial drug treatment. *Lancet Infectious Diseases*, 3: 28–32.

Ghosh AK, Takayama J, Rao KV, Ratia K, Chaudhuri R, Mulhearn DC, Lee H, Nichols DB, Baliji S, Baker SC, Johnson ME, Mesecar AD 2010. Severe acute respiratory syndrome coronavirus papain-like novel protease inhibitors): Design, synthesis, protein-ligand X-ray structure and biological evaluation. *Journal of Medicinal Chemistry*, 53(13): 4968–4979.

Ghosh AK, Takayama J, Aubin Y, Ratia K, Chaudhuri R, Baez Y, Sleeman K, Coughlin M, Nichols DB, Mulhearn DC, Prabhakar BS, Baker SC, Johnson ME, Mesecar AD 2009. Structure-based design, synthesis, and biological evaluation of a series of novel and reversible inhibitors for the severe acute respiratory syndrome - Coronavirus papain-like protease. *Journal of Medicinal Chemistry*, 52(16): 5228–5240.

Goh GB, Hadas NO, Vishnu A 2017. Deep learning for computational chemistry. *Journal of Computational Chemistry*, 38(16): 1291–1307.

Gorbatenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, Haagmans BL, Lauber C, Leontovich AM, Neuman BW, Penzar D, Perlman S, Poon LLM, Samborskiy D V., Sidorov IA, Sola I, Ziebuhr J 2020. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*, 5(4): 536–544.

Gorbatenya AE, Koonin E V. 1989. Viral proteins containing the purine NTP-binding sequence pattern. *Nucleic Acids Research*, 17(21): 8413–8438.

Groot RJ, Baker SC, Baric R, Enjuanes L, Gorbatenya AE, Holmes KV, Perlman S, Poon L, Rottier PJM, Talbot PJ, Woo PCY, Ziebuhr J 2020. Coronavirusidae family. International Committee on Taxonomy of Viruses (ICTV). Available from): https)://talk.ictvonline.org/ictv-
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Scaliant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/222/coronaviridae May 9, 2020).

Guang Xi X 2007. Helicases as Antiviral and Anticancer Drug Targets. Current Medicinal Chemistry, 14(8): 883–915.

Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z 2020. Cardiovascular Implications of Fatal Outcomes of Patients with Coronavirus Disease 2019 COVID-19. JAMA Cardiology, 5(7):811-818.

Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ 2003. Angiotensin-Converting Enzyme-2 ACE2)): Comparative Modeling of the Active Site, Specificity Requirements, and Chloride Dependence. Biochemistry, 42(45): 13185–13192.

Hao W, Wojdyla JA, Zhao R, Han R, Das R, Zlatev I, Manoharan M, Wang M, Cui S 2017. Crystal structure of Middle East respiratory syndrome coronavirus helicase. PLoS Pathogens, 13(6): e1006474.

Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S 2014. TMPRSS2 and ADAM17 Cleave ACE2 Differentially and Only Proteolysis by TMPRSS2 Augments Entry Driven by the Severe Acute Respiratory Syndrome Coronavirus Spike Protein. Journal of Virology, 88(2): 1293–1307.

Ho, Tin Yun, Shih Lu Wu, Jaw Chyun Chen, Chia Cheng Li, and Chien Yun Hsiang. 2007. Emodin Blocks the SARS Coronavirus Spike Protein and Angiotensin-Converting Enzyme 2 Interaction. Antiviral Research 74 (2): 92–101.

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 181(2): 271-280.

Hu S, Li S wen, Yan Q, Hu X peng, Li L yun, Zhou H, Pan L xin, Li J, Shen C pu, Xu T 2019. Natural products, extracts and formulations comprehensive therapy for the improvement of motor function in alcholic liver disease. Pharmacological Research, 150: 104501.

Hu TY, Frieman M, Wolfram J 2020. Insights from nanomedicine into chloroquine efficacy against COVID-19. Nature Nanotechnology, 15(1): 247–249.

Inglot AD 1969. Comparison of the antiviral activity in vitro of some non-steroidal anti-inflammatory drugs. The Journal of general virology, 4(2): 203–214.

Irie K, Nakagawa A, Fujita H, Tamura R, Eto M, Ikesue H, Muroi N, Tomii K, Hashida T 2020. Pharmacokinetics of Favipiravir in Critically Ill Patients with COVID-19. Clinical and Translational Science, 13(5): 880-885.

Irvani SSN, Golmohammadi M, Pourhoseingholi MA, Shokouhi S, Darazam IA 2020. Effectiveness of Interferon Beta 1a, compared to Interferon Beta 1b and the usual therapeutic regimen to treat adults with moderate to severe COVID-19): structured summary of a study protocol for a randomized controlled trial. Trials, 21(1): 473.
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Sciallant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

Islam MT, Sarkar C, El-Kersh DM, Jamaddar S, Uddin SJ, Shirpi JA, Mubarak MS 2020. Natural products and their derivatives against coronavirus): A review of the non-clinical and pre-clinical data. Phytotherapy Research, 34(10): 2471-2492.

Jaimes JA, André NM, Chappie JS, Millet JK, Whittaker GR 2020. Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop. Journal of Molecular Biology, 432(10): 3309-3325.

Jain RP, Pettersson HI, Zhang J, Aull KD, Fortin PD, Huitema C, Eltis LD, Parrish JC, James MNG, Wishart DS, Vederas JC 2004. Synthesis and evaluation of keto-glutamine analogues as potent inhibitors of severe acute respiratory syndrome 3CLpro. Journal of Medicinal Chemistry, 47:6113–6116.

Jasial S, Gilberg F, Blaschke T, Bajorath J 2018. Machine Learning Distinguishes with High Accuracy between Pan-Assay Interference Compounds That Are Promiscuous or Represent Dark Chemical Matter. Journal of Medicinal Chemistry, 61(22): 10255–10264.

Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C, Duan Y, Yu J, Wang L, Yang K, Liu F, Jiang R, Yang X, You T, Liu X, Yang X, Bai F, Liu H, Liu X, Guddar LW, Xu W, Xiao G, Qin C, Shi Z, Jiang H, Rao Z, Yang H 2020. Structure of Mpro from COVID-19 virus and discovery of its inhibitors. Nature, 582(7811): 289–293.

Kar S, Roy K 2013. How far can virtual screening take us in drug discovery? Expert Opinion on Drug Discovery, 8(3): 245–261.

Kawase M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S 2012. Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. Journal of virology, 86(12): 6537–45.

Keyaerts E, Vijgen L, Maes P, Neyts J, Ranst M Van 2004. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochemical and Biophysical Research Communications, 323(1): 264–268.

Kim DW, Seo KH, Curtis-Long MJ, Oh KY, Oh J-W, Cho JK, Lee KH, Park KH 2014. Phenolic phytochemical displaying SARS-CoV papain-like protease inhibition from the seeds of Psoralea corylifolia. Journal of Enzyme Inhibition and Medicinal Chemistry, 29(1): 59–63.

Kim, Dong Eon, Jung Sun Min, Min Seong Jang, Jun Young Lee, Young Sup Shin, Chul Min Park, Jong Hwan Song, et al. 2019. Natural Bis-Benzylsquinoquinoline Alkaloids-Tetrandrine, Fangchinoline, and Cepharanthine, Inhibit Human Coronavirus Oc43 Infection of Mrc-5 Human Lung Cells. Biomolecules, 9(11):696.

Kitchen DB, Decornez H, Furr JR, Bajorath J 2004. Docking and scoring in virtual screening for drug discovery): methods and applications. Nature Reviews Drug Discovery, 3(11): 935–949.

Kwong AD, Rao BG, Jeang KT 2005. Viral and cellular RNA helicases as antiviral targets. Nature Reviews Drug Discovery, 4(10): 845–853.

Lai MMC 1990. Corona Virus): Organization, Replication and Expression of Genome. Annual Review of Microbiology, 44(1): 303–303.
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Scaliant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

Lavecchia A 2015. Machine-learning approaches in drug discovery: methods and applications. Drug Discovery Today, 20(3): 318–331.

Lee H, Lei H, Santarsiero BD, Gatuz JL, Cao S, Rice AJ, Patel K, Szy pulpinski MZ, Ojeda I, Ghosh AK, Johnson ME. 2015. Inhibitor Recognition Specificity of MERS-CoV Papain-like Protease May Differ from That of SARS-CoV. ACS Chemical Biology, 10(6): 1456–1465.

Lee JM, Cho JB, Ahn HC, Jung W, Jeong YJ. 2017. A novel chemical compound for inhibition of SARS coronavirus helicase. Journal of Microbiology and Biotechnology, 27(11): 2070–2073.

Li F. 2005. Structure of SARS Coronavirus Spike Receptor-Binding Domain Complexed with Receptor. Science, 309(5742): 1864–1868.

Lee H, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, Zhang Q, Wu J. 2020a. Coronavirus infections and immune responses. Journal of Medical Virology, 92(4): 424–432.

Li G, Hu R, Zhang X. 2020b. Antihypertensive treatment with ACEI/ARB of patients with COVID-19 complicated by hypertension. Hypertension Research, 43(6):588-590.

Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin I, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Fang Z. 2020c. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. New England Journal of Medicine, 382(13): 1199–1207.

Li SY, Chen C, Zhang HQ, Guo HY, Wang H, Wang L, Zhang X, Hua SN, Yu J, Xiao PG, Li RS, Tan X. 2005. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. Antiviral Research, 67(1): 18–23.

Li W, Moore MJ, Vaslieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greeneugh TC, Choe H, Farzan M. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature, 426(6965): 450–454.

Lim HS, Im JS, Cho JY, Bae KS, Klein TA, Yeom JS, Kim TS, Choi JS, Jang IJ, Park JW. 2009. Pharmacokinetics of hydroxychloroquine and its clinical implications in chemoprophylaxis against malaria caused by plasmodium vivax. Antimicrobial Agents and Chemotherapy, 53(4): 1468–1475.

Lin, Longfei, Boran Ni, Hongmei Lin, Miao Zhang, Xuechun Li, Xingbin Yin, Changhai Qu, and Jian Ni. 2015. Traditional Usages, Botany, Phytochemistry, Pharmacology and Toxicology of Polygonum Multiflorum Thunb.: A Review. Journal of Ethnopharmacology, 159(15):158-183.

Lindenbach BD. 2013. Virion assembly and release. Current Topics in Microbiology and Immunology, 2(329): 199–218.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 23(1-3): 3–25.
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Scaliant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

Liu, Yue, Qi Wang, Jianbo Yang, Xiaohan Guo, Wenxi Liu, Shuangcheng Ma, and Shaoping Li. 2018. Polygonum Multiflorum Thunb.: A Review on Chemical Analysis, Processing Mechanism, Quality Evaluation, and Hepatotoxicity. *Frontiers in Pharmacology*, 9:364.

Luan J, Lu Y, Jin X, Zhang L 2020. Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochemical and Biophysical Research Communications*, 526(1): 165–169.

Lusci A, Pollastri G, Baldi P 2013. Deep Architectures and Deep Learning in Chemoinformatics): The Prediction of Aqueous Solubility for Drug-Like Molecules. *Journal of Chemical Information and Modeling*, 53(7): 1563–1575.

Mahévas M, Tran VT, Roumier M, Chabrol A, Paule R, Guillaud C, Fois E, Lepeule R, Szwebel TA, Lescure FX, Schlemmer F, Matignon M, Khellaf M, Crickx E, Terrier B, Morbieu C, Legendre P, Dang J, Schoindre Y, Pawlotsky JM, Michel M, Perrodeau E, Carlier N, Roche N, De Lastours V, Ourghanlian C, Kerneis S, Ménager P, Mouthon L, Audureau E, Ravaud P, Godeau B, Gallien S, Coster-Doet-Caluwme N 2020. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen): Observational comparative study using routine care data. *The British Medical Journal*, 369:m1844.

Makino S, Keck JG, Stohlman SA, Lai MM 1986. High-frequency RNA recombination of murine coronaviruses. *Journal of Virology*, 57(3): 729–737.

Marmor MF, Kellner U, Lai TYY, Melles RB, Mieler WF, Lum F 2016. Recommendations on Screening for Chloroquine and Hydroxychloroquine Retinopathy 2016 Revision). *Ophthalmoology*, 123(6): 1386–1394.

Marra MA, Jones SJM, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YSN, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL 2003. The genome sequence of the SARS-associated coronavirus. *Science*, 300(5624): 1399–1404.

McChesney EW 1983. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. *The American Journal of Medicine*, 75(1): 11–18.

Mihindukulasuriya KA, Wu G, St. Leger J, Nordhausen RW, Wang D 2008. Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *Journal of Virology*, 82(10): 5084–5088.

Molinari G 2009. Natural products in drug discovery): Present status and perspectives. *Advances in Experimental Medicine and Biology*, 655: 13–27.

Momokov G, Momekova D 2020. Ivermectin as a potential COVID-19 treatment from the pharmacokinetic point of view. *MedRxiv*. 

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Monteiro WM, Brito-Sousa JD, Baía-Da-Silva D, de Melo GC, Siqueira AM, Val F, Daniel-Ribeiro CT, Lacerda MVG 2020. Driving forces for covid-19 clinical trials using chloroquine): The need to choose the right research questions and outcomes. Revista da Sociedade Brasileira de Medicina Tropical 53: e20200155.

Neves BJ, Braga RC, Melo-Filho CC, Moreira-Filho JT, Muratov EN, Andrade CH 2018. QSAR-Based Virtual Screening: Advances and Applications in Drug Discovery. Frontiers in Pharmacology, 9: 1275.

Nicola Nosengo 2016. Can you teach old drugs new tricks? Nature, 534(7607): 314–316.

Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature Communications, 11(1): 1–12.

Paim FC, Bowman AS, Miller L, Feehan BJ, Marthaler D, Saif LJ, Vlasova AN 2019. Epidemiology of Deltacoronaviruses (δ-CoV) and Gammacoronaviruses (γ-CoV) in Wild Birds in the United States. Viruses 11(10): 897.

Pillaiyar T, Meenakshisundaram S, Manickam M 2020. Recent discovery and development of inhibitors targeting coronaviruses. Drug Discovery Today, 25(4): 668–688.

Pyrc K, Dijkman R, Deng L, Jebbink MF, Ross HA, Berkhout B, van der Hoek L 2006. Mosaic Structure of Human Coronavirus NL63, One Thousand Years of Evolution. Journal of Molecular Biology, 364(5): 964–973.

Ratia K, Kilianski A, Baez-Santos YM, Baker SC, Mesecar A 2014. Structural Basis for the Ubiquitin-Linkage Specificity and deISGylating Activity of SARS-CoV Papain-Like Protease. Rey FA Ed). PLoS Pathogens, 10(5): e1004113.

Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD 2018. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Research, 46(1): D624–D63.

Rodrigues T, Reker D, Schneider P, Schneider G 2016. Counting on natural products for drug design. Nature Chemistry, 8(6): 531–541.

Romano M, Ruggiero A, Squeglia F, Maga G, Berisio R 2020. A Structural View at SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. Cells, 9(5): 1267.

Rosenberg ES, Dufort EM, Udo T, Wilberschied IA, Kumar J, Tesoriero J, Weinberg P, Kirkenwood J, Muse A, Dehovitz J, Blog DS, Hutton B, Holgrave DR, Zucker HA 2020. Association of Treatment with Hydroxychloroquine or Azithromycin with In-Hospital Mortality in Patients with COVID-19 in New York State. Journal of the American Medical Association, 323(24): 2493-2502.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Peñaranda S, Bankamp B, Maher K, Chen M hsin, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TCT, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liifick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Günther S, Osterhaus ADHE, Drosten C, Pallansch MA, Anderson IJ, Bellini WJ 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science, 300(5624): 1394–1399.
Saurabh K, Rishi P, Rishi E, Kazi M 2013. Delayed onset chloroquine retinopathy presenting 10 years after long-term usage of chloroquine. *Middle East African Journal of Ophthalmology*, 20(1): 89.

Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R 2003. Effects of chloroquine on viral infections: An old drug against today’s diseases? *Lancet Infectious Diseases*, 3(11): 722–727.

Schoeman D, Fielding BC 2019. Coronavirus envelope protein: Current knowledge. *Virology Journal*, 16(69): 1–22.

Sepay N, Sepay N, Al Hoque A, Mondal R, Halder UC, Muddassir M 2020. In silico fight against novel coronavirus by finding chromone derivatives as inhibitor of coronavirus main proteases enzyme. *Structural Chemistry*, 5: 1-10.

Shadrick WR, Ndjoumou J, Kolli R, Mukherjee S, Hanson AM, Frick DN 2012. Discovering new medicines targeting helicases: Challenges and recent progress. *Journal of Biomolecular Screening*, 18(7): 761–781.

Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F 2020a. Cell entry mechanisms of SARS-CoV-2. *Proceedings of the National Academy of Sciences*, 117(21): 11727-11734.

Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F 2020b. Structural basis of receptor recognition by SARS-CoV-2. *Nature*, 581(7807): 221–224.

Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, MacKman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Science Translational Medicine*, 9(396): eaal3653.

Shen L, Niu J, Wang C, Huang B, Wang W, Zhu N, Deng Y, Wang H, Ye F, Cen S, Tan W 2019. High-Throughput Screening and Identification of Potent Broad-Spectrum Inhibitors of Coronaviruses. *Journal of Virology*, 93(112): e00023-19.

Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C 2020. Association of Cardiac Injury with Mortality in Hospitalized Patients with COVID-19 in Wuhan, China. *Journal of the American Medical Association*, 5(7):802-810.

Silva MF das GF, Fernandes JB, Forim MR, Vieira PC, de Sá ICG 2013. Alkaloids derived from anthranilic acid): Quinoline, acridone, and quinazoline. In: Ramawat KG, Mérillon J-M Eds, Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes. Springer Berlin Heidelberg, 715–859. https://doi.org/10.1007/978-3-642-22144-6_25.

Singh S, Carpenter AE, Genovesio A 2014. Increasing the Content of High-Content Screening): An Overview. *Journal of biomolecular screening*, 19(5): 640–650.

Singleton MR, Dillingham MS, Wigley DB 2007. Structure and Mechanism of Helicases and Nucleic Acid Translocases. *Annual Review of Biochemistry*, 76(1): 23–50.

Smit C, Peeters MYM, van den Anker JN, Knibbe CAJ 2020. Chloroquine for SARS-CoV-2): Implications of Its Unique Pharmacokinetic and Safety Properties. *Clinical Pharmacokinetics*, 59(6): 659–669.
Spiga O, Bernini A, Ciutti A, Chiellini S, Menciassi N, Finetti F, Causarono V, Anselmi F, Prischi F, Niccolai N 2003. Molecular modelling of S1 and S2 subunits of SARS coronavirus spike glycoprotein. Biochemical and Biophysical Research Communications, 310(1): 78–83.

Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, Gao GF 2016. Epidemiology, Genetic Recombination, and Pathogenesis of Coronavirus. Trends in Microbiology, 24(6): 490-502.

Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, Talavera-López C, Maatz H, Reichart D, Sampaiozitis F, Worlock KB, Yoshida M, Barnes JL 2020. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nature Medicine, 26(5): 681–687.

Takiuchi E, Barreiros MAB, Alfieri AF, Alfieri AA 2007. Identification of a mutation in the spike protein cleavage site in Brazilian strains of wild-type bovine coronavirus. Brazilian Journal of Microbiology, 38(4): 699–703.

Tang W, Cao Z, Han M, Wang Z, Chen J, Sun W, Wu Y, Xiao W, Liu S, Chen E, Chen W, Wang X, Yang J, Lin J, Zhao Q, Yan Y, Xie Z, Li D, Yang Y, Liu L, Qu J, Ning G, Shi G, Xie Q 2020. Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: Open label, randomised controlled trial. The British Medical Journal, 369: m1849.

Tanner JA, Zheng BJ, Zhou J, Watt RM, Jiang JQ, Wong KL, Lin YP, Lu IY, He ML, Kung HF, Kesel AJ, Huang JD 2005. The adamantane-derived bananins are potent inhibitors of the helicase activities and replication of SARS coronavirus. Chemistry and Biology, 12(3): 303–311.

Tanrikulu Y, Krüger B, Proschak E 2013. The holistic integration of virtual screening in drug discovery. Drug Discovery Today, 18(7-8): 358–364.

Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ 2000. A human homolog of angiotensin-converting enzyme): Cloning and functional expression as a captopril-insensitive carboxypeptidase. Journal of Biological Chemistry, 275(43): 33238–33243.

Uhal BD, Li X, Xue A, Gao X, Abdul-Haefz A 2011. Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1-7/ Mas axis. American Journal of Physiology - Lung Cellular and Molecular Physiology, 301(3): L269.

Valdés G, Neves LAA, Anton L, Corthorn J, Chacón C, Germain AM, Merrill DC, Ferrario CM, Sarao R, Penninger J, Brosnihan KB 2006. Distribution of angiotensin-(1-7. and ACE2 in human placentas of normal and pathological pregnancies. Placenta, 27(2-3): 200–207.

Vapnik VV 2000. The Nature of Statistical Learning Theory. 2nd ed. Springer, New York, 314 pp.

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD 2002. Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry, 45(12): 2615–2623.

Vickers C, Hales P, Kaushik V, Dick L, Gavvin J, Tang J, Godbouk K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P 2002. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. Journal of Biological Chemistry, 277(17): 14838–14843.
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rosseto, Rodrigo Sciallant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

Viegas C, Da Silva Bolzani V, Barreiro EJ 2006. OS produtos naturais e a química medicinal moderna. Quimica Nova, 29(2): 326–337.

Villegas, M., D. Vargas, J. D. Msonthi, A. Marston, and K. Hostettmann. 1988. Isolation of the Antifungal Compounds F卡尔indiol and Sarisan from Heteromorpha Trifoliata. Planta Medica, 54(1): 36–37.

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell, 181(2): 281-292.e6.

Wan Y, Shang J, Graham R, Baric RS, Li F 2020. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. Journal of Virology, 94(7): e00127.

Wang K, Chen W, Zhou Y-S, Lian J-Q, Zhang Z, Du P, Gong L, Zhang Y, Cui H-Y, Geng J-J, Wang B, Sun X-X, Wang C-F, Yang X, Lin P, Deng Y-Q, Wei D, Yang X-M, Zhu Y-M, Zhang K, Zheng Z-H, Miao J-L, Guo T, Shi Y, Zhang J, Fu L, Wang Q-Y, Bian H, Zhu P, Chen Z-N 2020. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. BioRxiv, 14: 988345.

Wang L-F, Lin Y-S, Huang N-C, Yu C-Y, Tsai W-L, Chen J-J, Kubota T, Matsuoka M, Chen S-R, Yang C-S, Lu R-W, Lin Y-L, Chang T-H 2015a. Hydroxychloroquine-Inhibited Dengue Virus Is Associated with Host Defense Machinery. Journal of Interferon & Cytokine Research, 35(3): 143–156.

Wang L, Yang R, Yuan B, Liu Y, Liu C 2015b. The antiviral and antimicrobial activities of licorice, a widely-used Chinese herb. Acta Pharmaceutica Sinica B 5: 310–315.

Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Zhou Z, Han L, Karapetyan K, Dracheva S, Shoemaker BA, Bolton E, Gindulyte A, Bryant SH 2012. PubChem’s BioAssay Database. Nucleic acids research, 40): D400–D412.

WHO WHO 2020. Coronavirus disease COVID-19. Pandemic. Available from): https://www.who.int/emergencies/diseases/novel-coronavirus-2019 May 8, 2020).

Woo PCY, Lau SKP, Chu C, Chan K, Tsoi H, Huang Y, Wong BHL, Poon RWS, Cai JJ, Luk W, Poon LLM, Wong SSY, Guan Y, Peiris JSM, Yuen K 2005. Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia. Journal of Virology, 79(2): 884–895.

Woo PCY, Huang Y, Lau SKP, Yuen KY 2010. Coronavirus genomics and bioinformatics analysis. Viruses, 2: 1805–1820.

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science, 367(6483): 1260–1263.

Wu CY, Jan JT, Ma SH, Kuo CJ, Juan HF, Cheng YSE, Hsu HH, Huang HC, Wu D, Brik A, Liang FS, Liu RS, Fang JM, Chen ST, Liang PH, Wong CH 2004. Small molecules targeting severe acute respiratory syndrome human coronavirus. Proceedings of the National Academy of Sciences of the United States of America, 101(27): 10012–10017.
Xudong X, Junzhu C, Xingxiang W, Furong Z, Yanrong L 2006. Age- and gender-related difference of ACE2 expression in rat lung. *Life Sciences*, 78(19): 2166–2171.

Yang H, Yang M, Ding Y, Liu Y, Lou Z, Zhou Z, Sun L, Mo I, Ye S, Pang H, Gao GF, Anand K, Bartlam M, Hilgenfeld R, Rao Z 2003. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, 100(23): 13190–13195.

Yang H, Xie W, Xue X, Yang K, Ma J, Liang W, Zhao Q, Zhou Z, Pei D, Ziebuhr J, Hilgenfeld R, Yuen KY, Wong L, Gao G, Chen S, Chen Z, Ma D, Bartlam M, Rao Z 2005. Design of Wide-Spectrum Inhibitors Targeting Coronavirus Main Proteases. *PLoS Biology*, 3: e324.

Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W, Liu D 2020. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clinical infections diseases*, 71(15):732-739.

Yin W, Mao C, Luan X, Shen D-D, Shen Q, Su H, Wang X, Zhou F, Zhao W, Gao M, Chang S, Xie Y-C, Tian G, Jiang H-W, Tao S-C, Shen J, Jiang Y, Jiang H, Xu Y, Zhang S, Zhang Y, Xu HE 2020. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science*, 368(6498):1499-1504.

Yu MS, Lee J, Lee JM, Kim Y, Chin YW, Jee JG, Keum YS, Jeong YJ 2012. Identification of myricetin and scutellarein as novel chemical inhibitors of the SARS coronavirus helicase, nsP13. *Bioorganic and Medicinal Chemistry Letters*, 22(12): 4049–4054.

Yu X-J, Luo C, Lin J-C, Hao P, He Y-Y, Guo Z-M, Qin L, Su J, Liu B-S, Huang Y, Nan P, Li C-S, Xiong B, Luo X-M, Zhao G-P, Pei G, Chen K-X, Shen X, Shen J-H, Zou J-P, He W-Z, Shi T-L, Zhong Y, Jiang H-L, Li Y-X 2003. Putative hAPN receptor binding sites in SARS_CoV spike protein. *Acta pharmacologica Sinica*, 24(6): 481–488.

Zaman S Bin, Hussain MA, Nye R, Mehta V, Mamun KT, Hossain N 2017. A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus*, 9(6):e1403.

Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W 2020. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *bioRxiv*, 919985.

Zhi K, Wang J, Zhao H, Yang X 2019. Self-assembled small molecule natural product gel for drug delivery: A breakthrough in new application of small molecule natural products. *Acta Pharmaceutica Sinica B*, 10(5): 913-927.

Zhong NS, Zheng BJ, Li YM, Poon LLM, Xie ZH, Chan KH, Li PH, Tan SY, Chang Q, Xie JP, Liu XQ, Xu J, Li DX, Yuen KY, Peiris JSM, Guan Y 2003. Epidemiology and cause of severe acute respiratory syndrome SARS. in Guangdong, People's Republic of China, in February, 2003. *Lancet* 362(9393): 1353–1358.

Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, Shi Z-L 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798): 270–273.
Starting Anti-COVID-19 Drug Discovery with Natural Products

Osmar Nascimento Silva, Bruno Junior Neves, Lucimar Rossetto, Rodrigo Scaliant Moura, Hamilton Napolitano, Wesley Brito, James Fajemiroye, Emerith Pinto, Pál Perjési, José Luís Martins

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W 2020. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine, 382(8): 727–733.

Zhu T, Cao S, Su P-C, Patel R, Shah D, Chokshi HB, Szukala R, Johnson ME, Hevener KE 2013. Hit identification and optimization in virtual screening: practical recommendations based on a critical literature analysis. Journal of medicinal chemistry, 56(17): 6560–72.

Ziebuhr J, Snijder EJ, Gorbalenya AE 2000. Virus-encoded proteinases and proteolytic processing in the Nidovirales. Journal of General Virology, 81(4): 853–879.

Zumla A, Chan JFW, Azhar EI, Hui DSC, Yuen KY 2016. Coronaviruses-drug discovery and therapeutic options. Nature Reviews Drug Discovery, 15: 327–347.

Iniciando a Descoberta de Drogas Anti-COVID-19 com Produtos Naturais

RESUMO

COVID-19 foi caracterizada como uma pandemia por sua rápida disseminação internacional e gravidade em março de 2020. A família Coronaviridae recebe esse nome devido à organização da glicoproteína de pico localizada no envelope, que se assemelha a uma coroa estelar quando observada ao microscópio. Os coronavírus sofrem mutações frequentes em seu genoma devido a erros cometidos pela RNA polimerase dependente de RNA (RdRp). O SARS-CoV-2 foi caracterizado por alta infectividade e transmissão pessoa a pessoa, com período de incubação de até quatorze dias. Atividades antivirais potentes de vários produtos naturais, como alcalóides, chalconas, triterpenóides, foram relatadas, mas com eficácia ou segurança não confirmadas na clínica, bem como nos mecanismos subjacentes completos. Além disso, CQ, HCQ e ivermectina, remdesivir, lopinavir, ritonavir, favipiravir e interferon pegulido com ribavirina foram testados para desenvolver uma terapia eficaz e uma vacina para tratar COVID-19. Este estudo investigou os efeitos antivirais dos produtos naturais contra SARS-CoV, HCoV-NL63, HCoV-229E e HCoV-OC43. Além disso, licorina, emodina, promazina, saikosaponinas B2, silvestrol, cefarantina, fangchinoline, tetrandrina, ácido cafeico, ácido clorogênico, ácido gálico e emetina são considerados compostos de sucesso importantes para a descoberta de drogas anti-SARS-CoV-2.

Palavras-Chave: Coronavírus; Descoberta de drogas; Produtos naturais; Transmissão.

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