Anurans against SARS-CoV-2: A review of the potential antiviral action of anurans cutaneous peptides

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ABSTRACT

At the end of 2019, in China, clinical signs and symptoms of unknown etiology have been reported in several patients whose sample sequencing revealed pneumonia caused by the SARS-CoV-2 virus. COVID-19 is a disease triggered by this virus, and in 2020, the World Health Organization declared it a pandemic. Since then, efforts have been made to find effective therapeutic agents against this disease. Identifying novel natural antiviral drugs can be an alternative to treatment. For this reason, antimicrobial peptides secreted by anurans' skin have gained attention for showing a promissory antiviral effect. Hence, this review aimed to elucidate how and which peptides secreted by anurans' skin can be considered therapeutic agents to treat or prevent human viral infectious diseases. Through a literature review, we attempted to identify potential antiviral frogs' peptides to combat COVID-19. As a result, the Magainin-1 and -2 peptides, from the Magainin family, the Dermaseptin-S9, from the Dermaseptin family, and Caerin 1.6 and 1.10, from the Caerin family, are molecules that already showed antiviral effects against SARS-CoV-2 *in silico*. In addition to these peptides, this review suggests that future studies should use other families that already have antiviral action against other viruses, such as Brevinins, Maculatins, Esculentins, Temporins, and Urumins. To apply these peptides as therapeutic agents, experimental studies with peptides already tested *in silico* and new studies with other families not tested yet should be considered.

1. Introduction

By the end of 2019, several cases of pneumonia of unknown cause were reported in Wuhan City, Hubei Province of China (Lu et al., 2020). Following a clinical sequencing of a group of patients, the SARS-CoV-2 virus was identified as the trigger of this clinical manifestation (Zhu et al., 2020). Among the symptoms, the disease caused by SARS-CoV-2 may vary from asymptomatic to severe acute respiratory syndrome (SARS) (Pan et al., 2019; Huang et al., 2020). The virus presented a high spread rate across the world when in March 2020 the World Health Organization (WHO) declared the novel coronavirus (COVID-19) outbreak a global pandemic. Today, there are almost 476 million confirmed cases and more than 6 million deaths caused by the coronavirus in the world (WHO, 2022). The increased number of COVID-19 cases along with the emergence of new variants turned into an urgent need to find an effective cost-effective solution to this disease.

Coronaviruses (CoVs) are a virus group of single-stranded ribonucleic acid (RNA) positive-sense, enveloped, belonging to the *Coronaviridae* family, and originally infect birds and mammals (V'kovski et al., 2021). These viruses have been spread in the human population and cause respiratory illnesses similar to the common cold (Ahmed et al., 2020). Nevertheless, some infections caused by zoonotic coronaviruses have led to lethal epidemics, such as SARS-CoV (severe acute respiratory syndrome) in 2003 (see Parashar and Anderson, 2004), MERS-CoV (Middle East Respiratory Syndrome) in 2012 (see Park et al., 2018), and the SARS-COV-2 pandemic in 2019 (see Hu et al., 2021).

The emergence of zoonotic viruses, such as SARS-CoV-2, supports the idea that these viruses, especially RNA viruses, are at higher risk than other pathogens for developing diseases in humans because of their high mutation rates (Cleaveland et al., 2001). Due to this high mutation rate, genetic instability has been considered a challenge to develop effective vaccines against RNA viruses (Forni et al., 2021). In addition, there is also the risk of reinfection (Coutinho et al., 2021), the emergence of new variants of SARS-CoV-2 affecting new patients and infecting those who has already been vaccinated (Koyama et al., 2020). In this scenario, the constant spreading risk and the chances of new contagious viral diseases

require studies to find alternative treatments to the disease.

It is well known that several anurans' species secrete through the skin surface substances with biological activities which diverse functions, having alternative applications as anti-cancer, anti-inflammatory, anti-diabetes, and antiviral agents (Conlon et al., 2014). Therefore, the interest in these secretions due their potential has been increased for the development of new drugs. Among these substances, are peptides known to act as a defense substance for animals with antifungal and antibacterial actions capable to permeable into mammalian cells (Conlon, 2011). Besides, these peptides are potent antivirals, directly inactivating viral particles or interfering with the virus's reproductive cycle (Mulder et al., 2013). The anurans' skin peptides are stored in granular glands, mainly located in the skin dorsal region (Conlon et al., 2019), and become potential candidates for developing new antiviral agents.

Amphibians are a vast group with 8434 species (88% belonging to the order Anura) and approximately 150 new species are discovered each year (AmphibiaWeb, 2022). These animals are known for their ability to exploit both aquatic and terrestrial habitats and have a skin that plays vital physiological functions (such as water absorption, osmoregulation, and respiration; Duellman and Trueb, 1986) and fundamental roles in their survival in these habitats (Farquhar and Palade, 1965; Elkan et al., 1968; Lillywhite et al., 2006; Suzuki et al., 2007). Therefore, the skin is a sensitive and selective interface between the internal and external environment of these organisms and is routinely exposed to several environmental factors. To keep the skin healthy and functional, these animals have developed, for example, defense mechanisms against pathogens and recovery from dermal wounds. Unlike other vertebrates, amphibians can heal deep wounds without scarring and regenerate epidermal glands (Yokoyama et al., 2018). In addition, several peptides secreted by the skin have been described with anti-pathogenic action (Chinchar et al., 2004; Calhoun et al., 2016). Due to the richness of species and characteristics related to these animals, the skin of amphibians becomes an important target for studies involving peptides and their possible therapeutic applications.

Frog skin is by far the most abundant source of animal AMPs, with 1057 peptides out of 2482 listed in the Antimicrobial Peptides Database (last updated: Feb 28, 2022). Considered a source of antimicrobial peptides, many studies have explored the therapeutic potential of peptides, which are naturally abundant in anurans' skin. In the last 20 years, some studies have explored the role of these peptides as antivirals. Dermaseptins S1–S5 peptides, for example, show antiviral activity against herpes simplex virus type I and HIV-1 at micromolar doses (Lorin et al., 2005). Curiously, frogs' peptides correspond to ~60% of the anti-HIV-1 AMPs from the animal kingdom already described (Wang, 2012). Some recent studies have been projected bioinformatics interactions between anuran skin peptides sequences against SARS-CoV-2 searching for possible antiviral action against this new coronavirus (Fakih, 2020; Fakih et al., 2020; Liscano et al., 2020). The use of computational tools have been essential to better understand the viral infection, the immune response, and to development new treatments and vaccines candidates efficiently against SARS-CoV-2 (Lee et al., 2021b; Sharma et al., 2021).

Bearing that in mind, the present study aimed to elucidate how the peptides secreted by the anurans' skin can be considered therapeutic agents for the treatment or prevention of infectious viral diseases, especially Covid-19. Also, we describe the knowledge about the skin peptides of frogs, their antiviral action, and the main antiviral peptides already studied against Covid-19. Furthermore, we report the mode of infection of SARS-Cov 2 and their interaction with Caerin, Magainin, and Dermaseptin peptides secreted by the anurans' skin. Finally, we describe the use of *in silico* studies as a tool to find new possible antiviral peptides and elucidate some families of peptides that can be used for further studies aiming at an antiviral action.

2. Overview of the peptides secreted by anurans skin

The anurans skin has an intense chemical diversity and versatile functions, which are required for survival. The skin is highly specialized supporting several physiological functions as respiration, osmoregulation, and thermoregulation (König et al., 2015) . Also, the anuran skin has a defense function, releasing substances to protect against microorganisms, and control the skin infestation by arthropods i.e., parasitic mites or insect larvae (Xu and Lai, 2015; Cardoso et al., 2013). The secretions present in the anurans' skin have great chemical diversity and due to their properties and biosynthesis pathways. There are increasing indications of intra-individual variation in secreted products between different granular gland types (Maciel et al., 2003), results that support the remarkable plasticity and adaptive value of amphibian skin. In the last few years, the bioactive components of these secretions from the amphibian skin, mainly biologically active peptides, have been extensively studied.

Amphibian skin has granular (serous) and mucosal glands (Duellman and Trueb, 1986; Wells, 2007) that are distributed over the dorsal side. These glands communicate directly with the surface through secretory ducts. Through these ducts, the mucosal glands continually release small amounts of mucopolysaccharides to keep the skin well-moisturized (Mills and Prum, 1984). The granular glands, on the other hand, release venous and toxic compounds through discharges induced by various stimuli, for example, by stress generated in a predatory attack (König et al., 2015). The granular gland is surrounded by smooth muscle (Noble and Noble, 1944) or myoepithelium (Dockray and Hopkins, 1975). These glands respond to α-adrenergic or nerve stimulation by contracting these muscles and, upon muscle contraction, the expulsion of the glandular contents occurs (Hoffman and Dent, 1977; Dockray and Hopkins, 1975). In the laboratory, stimulation to release these secretions mimics the environmental stress response through mild electrical stimulation or norepinephrine injections (Pál et al., 2006).

It is known that anuran cutaneous secretion is a complex mixture of biologically active compounds, including biogenic amines, alkaloids, and peptides (Simmaco et al., 1998). Peptides are stored in the skin's granular glands and can be released in high concentrations when the amphibian is stressed or injured. Amphibians are famous for their abundance of biologically active peptides (Conlon et al., 2004; Mangoni, 2006; Xu and Lai, 2015; Bowie et al., 2012). There are more than 1000 peptides described in the amphibian skin, obtained mainly from frog species belonging to the *Ascaphidae, Alytidae, Bombinatoridae, Hylidae, Hyperoliidae, Leptodactylidae, Myobatrachidae, Pipidae and Ranidae* families (Conlon, 2011; Xu and Lai, 2015; Wang and Epand, 2016; APD, 2022) . The structure of these peptides contains between 5 and 63 amino acids (~99.9% of these frog peptides are less than 50 amino acid residues), and a comparison of their amino acid sequence reveals the lack of any conserved domains associated with biological activity (APD, 2022; Wang, 2020a). Frog skin peptides, with few exceptions, are cationic due to the presence of various lysine and arginines residues, and predominantly contain the amino acids leucine and isoleucine (Conlon and Sonnevend, 2010). They lack a stable conformation in aqueous solutions, but adopt an amphipathic α-helical structure in phospholipid vesicles in the environment or a membrane-mimetic solvent, such as 50% trifluoroethanol–water (Wang et al., 2016; Pantic, 2017). These peptides belong to the groups of myotropical peptides, opioid peptides, corticotropin-releasing peptides, neuropeptides, antioxidant peptides, insulin-releasing peptides, mast cell degradation/histamine-releasing peptides, wound-healing peptides, antimicrobial peptides, antitumor peptides, antiparasitic peptides, antiviral peptides, among others (Xu and Lai, 2015).

3. Antimicrobial peptides (AMPs) and antiviral peptides (AVPs)

The first and widely studied anuran peptides group is the antimicrobial peptides (AMPs) or also called host defense peptides (HDPs),

being particularly abundant in skin secretions (Simmaco et al., 1998). These peptides have several functions and targets in terms of biological activities (Wang and Epand, 2016), from antimicrobial, antiviral, and antifungal agents (Reddy et al., 2004; Mello et al., 2011; Torcato et al., 2013a, 2013b) to response modulation immune (Silva et al., 2012). Regarding the latter, the rapid and non-specific interaction of AMPs with the membrane lipids of microbial targets results in the death of the pathogen (Arouri et al., 2009). Pathogen death is caused by the presence of many cationic and hydrophobic amino acid residues in the peptide structure. For this purpose, the AMPs are electrostatically attracted by the anionic membrane of the pathogen, breaking the lipid structures and promoting the permeation of the microorganism and, consequently, its death (Huang et al., 2013). Besides, there is a small chance of developing pathogens resistance (Fernebro, 2011), since the required alterations in the cell membrane for this purpose involve a great biological effort by the cellular machinery (Chen et al., 2014). Recent studies have indicated the antimicrobial activity of AMPs. However, it should be noted that some studies provide evidence that the activity may also be due to the interactions of these peptides with intracellular targets (Otvos, 2005).

AMPs, in addition to being involved in the immune response of these animals, can suppress the damaging inflammatory response from exposure to pathogens (Haney and Hancock, 2013; Mansour et al., 2014). This organization of the amphibian immune system is very similar to mammals, as they share similar functions related to innate and adaptive immunity (Fremont-Rahl, 2011; Colombo et al., 2015). For example, some genes that code pro-inflammatory cytokines, such as interleukin-6 (IL-6), are present in the genome of *Xenopus tropicalis* frogs, and there are also reports of skin macrophages activation in response to extracellular pathogens (Robert and Otha, 2009; Fremont-Rahl, 2011). Temporin A, a peptide secreted by the granular glands of amphibians, has been observed to promote by chemoattraction an influx of phagocytes, including macrophages, monocytes, and neutrophils, to the site exposed to the pathogen (Chen et al., 2004). The microbicide actions of frog skin peptides against multidrug-resistant pathogens have attracted considerable attention, and several synthetic analogs with increased antimicrobial potential, decreased toxicity, and a longer circulating half-life have been evaluated.

Most of the anuran's peptides consist of two distinct classes: linear helical peptides or peptides with cysteine with one disulfide bridge forming the C-terminal loop (Rinaldi, 2002). A common structural feature of AMPs is the clustering of hydrophobic and cationic residues on opposite sides of the peptide helix, making these molecules amphipathic (Nicolas and Mor, 1995) and it is these positively charged regions that bind to the major groups of negatively charged bacterial membranes (Matsuzaki, 1999).

Currently, more than 178 families of antimicrobial peptides (AMPs) from amphibian skin have been identified (Xu and Lai, 2015; Shartouny and Jacob, 2019). Amphibians belonging to different families, genera, or species could store a distinct range of peptides belonging to different AMP families (Ladram and Nicolas, 2016). AMPs are mainly distributed among the following families: Magainins, Dermaseptins, Brevinins, Esculentins, Temporins, Caerins, Ranalexins, Ranatuerins, Palustrins, Tigerinins, Japonicins, Maculatins and Nigrocins (Conlon et al., 2004). Magainins were the first AMPs identified in amphibians and are present in the African clawed frog *Xenopus laevis* (Zasloff, 1987). These peptides have been shown to have antibacterial, antiviral, antifungal, and anti-parasitic activities (Zasloff, 2002). In addition to Magainins, Dermaseptins are a superfamily of peptides found in several frog species, including species from the *Hylidae* and *Ranidae* families (Nicolas and El Amri, 2009). Similar to Megainins, Dermaseptins have been shown to have antimicrobial activity against a wide variety of bacterial pathogens involved in human disease, including *Pseudomonas, Salmonella, Staphylococcus, Escherichia*, and *Enterobacter* species. Dermaseptin-S3 and Magainin-2 are two amphibian-derived cationic peptides that interacted with DNA *in vitro* (Lohner and Leber, 2016) and both interfered with the integrity of *Saccharomyces cerevisiae* DNA *in vivo* (Morton et al., 2007).

That infers that both peptides can pass through the cytoplasmic membrane of bacteria cells, causing damage to cell membrane and DNA, as well as possessing intracellular targets. Moreover, has been revealed a possible role for Dermaseptins in the treatment of viral infections, such as HIV-1 (VanCompernolle et al., 2005).

It is well known that host defense peptides can also be immunomodulatory and inflammatory modulators (Epand et al., 2016). Consequently, it is expected that these agents may also have antiviral activity been a promising alternative in the design of therapeutics to control viral diseases (Field and De Clercq, 2004). In addition to stimulating inflammation and the immune system, the antiviral peptides (AVPs) are a potential resource for the development of new potent therapeutics for preventing or treating viral infection, having a broad-spectrum antiviral activity.

Global morbidity and mortality from viral infections are increasing, bringing attention to the importance of developing effective therapies against viruses (Barlow et al., 2014). Due to the emergence of new treatments and vaccines to combat these infections, antimicrobial peptides that have antiviral mechanisms have gained attention. Currently, there are 1135 active peptides from amphibians (1057 from frogs and 74 from toads; APD, 2022), with ~15% of AMPs showing antiviral activities against enveloped viruses (Mishra et al., 2017). Antiviral peptides (AVPs) have a wide activity range against human and animal's infectious viruses (see Vilas Boas et al., 2019). The AVPs are upregulated during infection by multiple pathogens *in vivo* via the immunological pathway (Alexander et al., 2018; Mookherjee et al., 2020). Based on available data, AVPs enrichment in organisms increases innate and adaptive immunity that drives antiviral activity. The antiviral activity of AMPs has been demonstrated against enveloped RNA and DNA viruses (Vilas Boas et al., 2019). Therefore, being a source of several peptides already reported (Conlon and Sonnevend, 2010), anurans' AVPs have received considerable attention as therapeutic agents against infectious viral pathogens such as dengue virus (DENV), Zika virus (ZIKV), and recently, SARS-CoV-2 (Ahmed et al., 2019).

4. Anurans AVPs

The main benefit of using AVPs as therapeutics is their hydrolysis by peptidases present in the body, which prevents its accumulation in specific organs and minimizes the toxic side effects (Ali et al., 2013). The AVPs can be obtained through different approaches such as computational approach (molecular docking, peptidomimetics), biological source (phage, mRNA, ribosome, yeast, and bacterial displays), and natural sources (bacteria, plants, marine organisms, arthropods, mammals, and amphibians) (Agarwal and Gabrani, 2021). AMPs and AVPs are usually derived from natural sources but they can be readily modified by adding non-natural amino acids or chemical groups to further enhance their stability and activity (Gentilucci et al., 2010).

The anuran antiviral peptides studies are recent and still lack more information. However, most AVPs already described derived from anurans are cationic, amphipathic, and α-helical (AlbiolMatanic and Castilla, 2004; Lorin et al., 2005; Bergaoui et al., 2013; Marcocci et al., 2018; Monteiro et al., 2018). Therefore, these characteristics may be structural features that can identify anuran peptides as antiviral agents in future studies. Moreover, there is no evidence of great physicochemical differences between AMPs and other AVPs (Wang et al., 2017). However, data show that hydrophobicity seems to be a critical property for those peptides with activity against enveloped viruses (Badani et al., 2014; (Wang et al., 2017) ; Vilas Boas et al., 2019). Another characteristic of frog AVPs is their modes of action. Although they have diverse modes of action, most can inhibit the virus at the cell entry step, the earliest phase of infection in the viral life cycle. This inhibition may be by interfering in the cell-to-cell spread of the virus, targeting viral envelopes, or targeting cell receptors (Agarwal and Gabrani, 2021). The AVPs also have a virucidal activity for some viruses, mainly attacking enveloped viruses (Monteiro et al., 2018).

Several studies have reported the antiviral action of anurans peptide secretions against a range of viruses that cause human diseases, such as HSV-1, HSV-2, and HIV-1 (Yasin et al., 2000; Belaid et al., 2002; Albiol Matanic and Castilla, 2004; Chinchar et al., 2004; Lorin et al., 2005; Mulder et al., 2013; Table 1). Knowing which antiviral peptides were previously described against human viruses and their actions modes brings new prospects for studies in this area. For example, we can use anuran's peptides that have not already been tested against antiviral diseases to evaluate their antiviral capacity. Thus, these studies can elucidate new therapeutic treatment agents and possible combinations of peptides to improve or enhance this mechanism. In this line, we will discuss which peptides secreted by the anurans' skin with antiviral activity on human viruses have already been described, and which are their action modes.

4.1. General mechanism of action

The mechanisms of antiviral drugs action are mainly acting on the virus itself or acting on the host. Also, antiviral drugs act on various enzymes related to transcription and replication to destroy the viral pathogen or inactivate its infectivity (Vilas Boas et al., 2019; Lou et al., 2014). Although viral entry is the favored target for AVPs, the AVPs have broad antiviral activity through different mechanisms of action.

Most AVPs block viral entry by one of the next mechanisms: interaction with heparan sulfate, blocking of cell-to-cell spread, interaction with specific cellular receptors, interaction with viral glycoproteins, membrane or viral envelope interaction (Jenssen et al., 2006). The antiviral peptides can block, for example, virus fusion or its entry into host cells, preventing viral propagation through gene expression suppression or an immunomodulatory mechanism (Jenssen et al., 2006; Mulder et al., 2013). AVPs block virus entry into the host cell through interaction with glycosaminoglycan molecules, an important molecule for virus-host cell binding (Andersen et al., 2003; Jenssen et al., 2004). Also, most AVP inhibit enveloped viruses' entry by physicochemical interaction with hydrophobic membrane–protein interfaces due to the positively charged residues of AVPs. Due to this interaction, electrostatic interaction between them and negatively charged cell surface molecules, such as heparan sulfate – which consists of glycosaminoglycan molecules that are strongly related to viral binding (Mettenleiter, 2001) can occur. The Magainin peptide has this action system, binding to glycosaminoglycans to prevent viral host cells attachment (Jenssen et al., 2006).

AVPs can also interact with specific cell receptors to inhibit viral entry into hosts. The peptide polyfemusin T22 has been shown to have potent antiviral activity against HIV-1, where the peptide binds to a CXCR4 chemokine receptor, a co-receptor for HIV-1 entry into T cells (Nakashima et al., 1992; Tamamura et al., 1998). Another mechanism of antiviral action of peptides is by interfering with the ATPase enzyme activity, interrupting the fusion process in the cell membrane (Albiol Matanic and Castilla, 2004). AVPs further protect the host cell from different viral infections by interacting with the virus envelope or by breaking the viral envelope/capsid (Migliolo et al., 2012; Teixeira et al., 2013). Dermaseptin, for example, has anti-HIV activity as it interacts directly with the viral particle, breaking the virus membrane (Lorin et al., 2005).

Another mechanism of AVPs action is inhibition of viral replication or protein synthesis to prevent viral replication in the host cell. Ribosomal inactivation by the pokeweed antiviral protein (PAP) peptide, for example, prevented tobacco mosaic virus (TMV) infection by blocking viral protein synthesis (Taylor et al., 1994). Lastly, there is the AVPs antiviral mechanism related to the suppression of viral gene expression and immunity modulation, a mechanism used by melittin and cecropin A peptides, which inhibit HIV-1 infection by suppressing the virus's gene expression (Wachinger et al., 1998). The omBD peptide, isolated from rainbow trout (*Oncorhynchus mykiss*), showed an immunomodulatory mechanism because inhibited the infection caused by the viral hemorrhagic septicemia virus (HVSM) by positively regulating the expression of the mx1 gene (Falco et al., 2007).

The following section elucidate anurans AVPs with potential activity against human RNA viruses. Based on these action modes already described, how these AVPs can interfere in the SARS-CoV-2 life cycle is shown in Fig. 1. As frogs have a huge variety of active peptides, new

Table 1
Anuran AVPs against human virus: AVP, peptide family, frog species, sequence, type virus and reference.

AVP	Peptide family	Frog species	Sequences	Virus	References
Caerin 1.1	Caerin	Litoria splendida (Pelodryadidae) *Litoria caerulea* (Pelodryadidae)	GLLSVLGSVAKHVLPHVVPVIAEHL	HPV and HIV	Stone et al., 1992 VanCompernolle et al., 2005 VanCompernolle et al., 2015 Ni et al., 2018
Caerin 1.9		*Litoria chloris* (Pelodryadidae)	GLFGVLGSIAKHVLPHVVPVIAEKL		Pan et al., 2019 Ni et al., 2020
Dermaseptin S4	Dermaseptin	*Phyllomedusa* (Phyllomedusidae)	ALWMTLLKKVLKAAAKAALNAVLVGANA	HIV and HSV	Lorin et al., 2005 Bergaoui et al., 2013
Dermaseptin S1			ALWKTMLKKLGTMALHAGKGAIUIDTISQGTQ	HSV and DENV	Savoia et al., 2010 Cardoso et al., 2013
Esculentin - 1GN	Esculentin	*Sylvirana guentheri* (Ranidae)	GLFSKKGGKGGKSWIKGVFKGIKGIGKEVGGD VIRTGIEIAACKIKGEC	H5N1	Yang et al., 2021
HS-1	Unclassified	*Hypsiboas semilineatus* (Hylidae)	H-FLPLILPSIVTALSSFLKQG-OH	DENV 2 and DENV 3	Monteiro et al., 2018
Maculatin 1.1	Maculatin	*Litoria genimaculata* (Pelodryadidae)	GLFGVLAKVAAHVVPAIAEHF	HPV and HIV	Rozek et al., 1998 VanCompernolle et al., 2005
Magainin-1	Magainin	*Xenopus* (Pipidae)	GIGKFLHSAGKFGKAFVGEIMK	HSV	Egal et al., 1999 Albiol Matanic and Castilla, 2004
Magainin-2			GIGKFLHSAKKFGKAFVGEIMNS		
Temporin-SHa	Temporin	*Pelophylax saharicus* (Ranidae)	FLSGIVGMLGKLF	HSV-1	Roy et al., 2019
Temporin-Tb		*Rana temporaria* (Ranidae)	LLPIVGNLLKSLL		Marcocci et al., 2018
Urumin	Urumin	*Hydrophylax bahuvistara* (Ranidae)	IPLRGAFINGRWDSQCHRFSNGAIACA	H1N1	Holthausen et al., 2017
Yodha	Brevinin	*Indosylvirana aurantiaca* (Ranidae)	SMLLLFFLGTISLSLCQDDQERC	DENV and ZIKV	Lee et al., 2020

AVP: antiviral peptide; DENV: dengue virus type; H1N1: influenza A virus subtype H1N1; H5N1: influenza A virus subtype H5N1; HIV: human immunodeficiency virus; HPV: human papillomavirus; HSV: herpes simplex virus; ZIKV: Zika virus
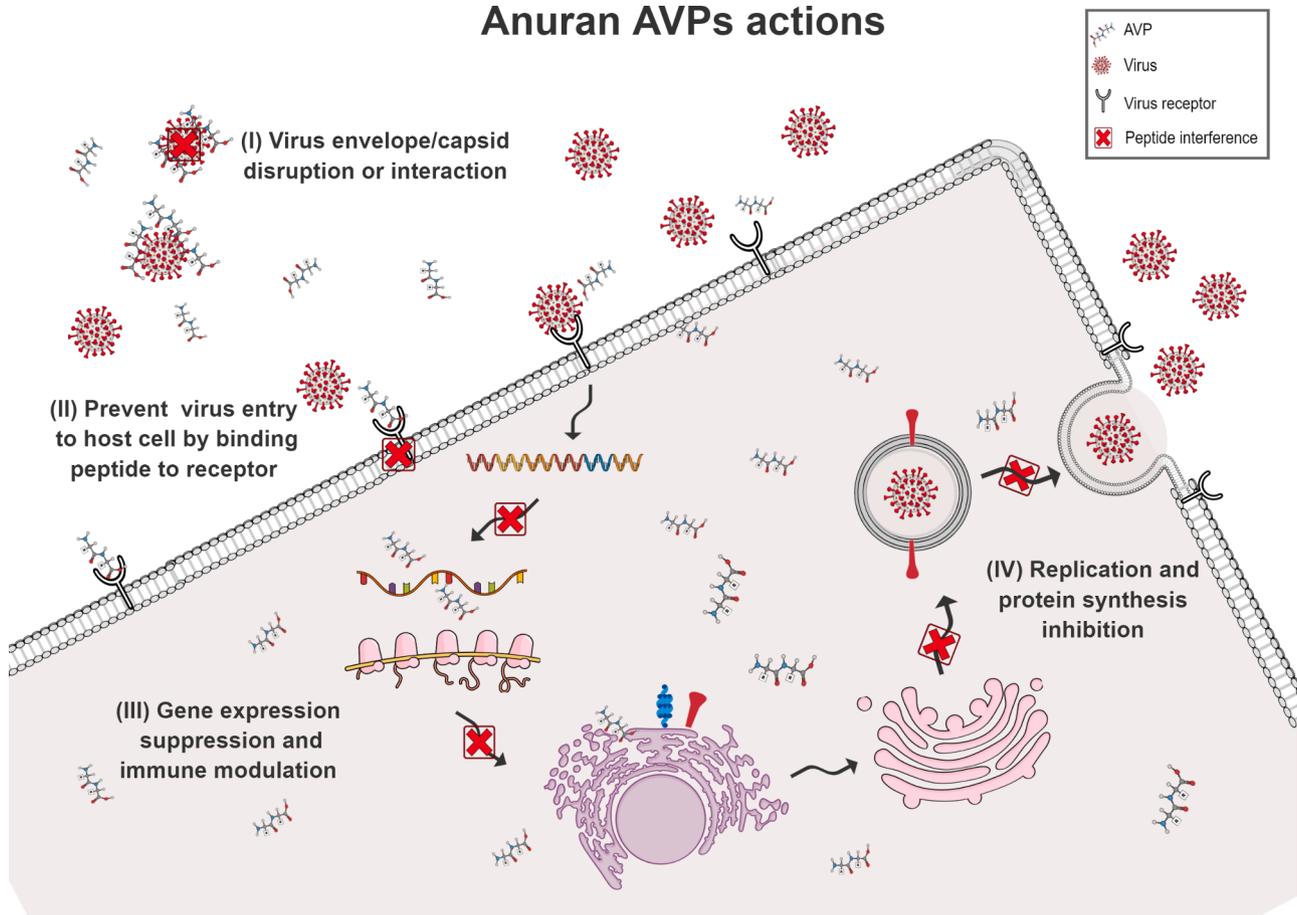
Anuran AVPs actions

Fig. 1. Potential action mechanisms of anurans' AVPs against SARS-CoV-2 – (I) virus envelope/capsid disruption or interaction, (II) prevent virus entry cell by binding peptide to receptor (III) suppression of virus gene expression and immune modulation, and (IV) avert seeding of virus within cell by inhibiting their replication or protein synthesis.

studies through scientific experiments, such as in silico analysis, could discover potential modes of peptides actions interfering in the virus cycle to prevent or stop the viral replication.

5. Anurans peptides against human viruses

5.1. Brevinins

Brevinins are one of the most omnipresent anurans antibacterial peptides and have two subfamilies: Brevinin-1, with 24 amino acid residues, and Brevinin-2, with 33 amino acid residues (Peschel et al., 2006; Chan et al., 2006). Brevinin-1 was originally isolated from *Rana limnocharis* skin and has been shown a broad antimicrobial action (Wang et al., 2016). Brevinin-2, isolated from Japanese frog *Rana brevipoda porsa* skin (Morikawa et al., 1992), has a wide distribution in Asian and European ranid frog species (Park et al., 1994, 2001; Kim et al., 2001) and European frogs (Simmaco et al., 1994, 1998).

The antiviral action of Brevinins was described recently. The peptide called Yodha, belonging to the Brevinin family, contains 23 amino acids (SMLLLFFLGTISLSLCQDDQERC) and has antiviral action against Dengue virus and Zika virus (Lee et al., 2021b). This peptide, which was isolated from *Indosylvirana aurantiaca* skin, has its amino acids with hydrophobic side chains dominating almost half of the N-terminal region, and the C-terminus contains three negatively charged amino acids (Lee et al., 2021a). According to Lee et al. (2021b), its antiviral action essentially consists of inducing the virus lysis, probably acting on a target inside the viral envelope. The peptide destabilizes the viral particle by integrating with the viral lipid bilayer using the hydrophobic

N-terminus and rapidly neutralizing the virus (within 5 min). Moreover, the Yodha peptide acts on all Zika virus strains and is non-toxic to human erythrocytes.

5.2. Caerins e maculatins

The three-dimensional structure of Caerins consists of two well-defined amphipathic alpha-helices separated by a short flexible region with a less defined helix. Has positive charges between +1 and +3, 53–56% hydrophobicity range, and length from 24 to 25 amino acid residues (Wong et al., 1997; Pukala et al., 2004). Caerin 1.1 (GLLSVLGSVAKHVLPHVVPVIAEHL) and Caerin 1.9 (GLFGVLGSIAKHVLPHVVPVIAEKL) were originally isolated from Australian tree frog skin of the genus *Litoria* and they have antiviral action by inhibiting HIV-infected T cells, in concentrations that are not toxic to T cells, and can inhibit the transfer of HIV from dendritic cells (DCs) to T cells (VanCompernolle et al., 2015, 2005). Recently, Caerin 1.1, peptides found in *Litoria caerulea*, and Caerin 1.9 found in *Litoria chloris*, have been shown additive effects against human papillomavirus (HPV)-transformed tumor cells (Ni et al., 2020). Both peptides also have potential action to increase the efficacy of a therapeutic vaccine against HPV-related diseases (Ni et al., 2018; Pan et al., 2019).

Maculatin 1.1 (GLFGVLAKVAAHVVPAIAEHF), secreted by Australian frog *Litoria genimaculata* skin (Rozek et al., 1998), has antiviral activity against HPV. With a sequence similar to Caerin 1.1, Maculatin 1.1. inhibited HIV infection without adversely affecting T cell viability, but it needs a higher concentration to promote inhibition compared to Caerin 1.1. and Caerin 1.9 (VanCompernolle et al., 2005).

5.3. Dermaseptins

Dermaseptins are a large family of AMPs and were first identified from the skin of the frog *Phyllomedusa sauvagii* (Mor et al., 1991). Dermaseptins currently represent the largest family of anuran peptides (König et al., 2015), with more than 70 peptides identified from 15 *Phyllomedusidae* species' skin (Nicolas and Ladram, 2013). They are α-helical peptides, linear polycationic, and very heterogeneous in length, containing 21 and 33 amino acid residues (Lequin et al., 2006). We can arrange Dermaseptin into two subclasses, as they have a highly conserved n-terminal sequence. The first subclass displays Ala1-X-Trp-Lys-YX-Leu-Lys8 (Dermaseptin-1, X represents a hydrophobic side chain and Y one side polar chain or aspartate). The second subclass displays a Gly1 followed by an alanine-rich motif in the intermediate region (-Ala-Ala/Gly-Lys/Gln-Ala-Ala-Leu-Gly/Asn-; Dermaseptin-2) (König et al., 2015).

Dermaseptins and their analogs are known to have lytic activity *in vitro*, antimicrobial ability with different efficiencies, and cytolytic activities against a wide range of free-living microorganisms (Nicolas and El Amri, 2009). Most of these peptides have an effect, at micromolar doses, against a broad spectrum of microorganisms. Furthermore, when Dermaseptins are combined with other antibiotic molecules or AMPs, their antimicrobial potency may increase 100-fold (Mor and Nicolas, 1994; Giacometti et al., 2006).

Dermaseptins S4 and their analogs from *Phyllomedusa sauvagii*, for example, are effective against the Herpes simplex-2 virus (HSV-2, Bergaoui et al., 2013). Lorin et al. (2005) demonstrated that Dermaseptin S4 could be a potential candidate for anti-HIV as it was capable to disrupt viral particles before infection, reducing HIV-1 binding to human endometrial cells (HEC-1) and HIV-1 transcytosis through a compact HEC-1 monolayer. Dermaseptin S1 exhibited antiviral activity against herpes simplex virus type I (HSV-1; Savoia et al., 2010) interrupting virion integrity, and against Dengue virus *in vitro* interfering with the viral replication cycle (Cardoso et al., 2013).

All Dermaseptins, except DRS-S4, are not or are lower toxic against mammalian cells (Ladram and Nicolas, 2016). Because of this, these molecules become interesting as possible therapeutic agents in combating microorganisms or as additional protection against infections, including viral infections.

5.4. Esculentins

Esculentins were first isolated from the skin secretion of the European frog *Rana esculenta,* and one of the first peptides families characterized (Morikawa et al., 1992; Simmaco et al., 1993, 1994; Basir et al., 2000; Ali et al., 2002). Afterward, Esculentins were identified from the skin of 13 different ranids (Simmaco et al., 1994; Xu and Lai, 2015). These peptides have about 46 amino acids, with a disulfide bridge at the C-terminus, and only change one or two amino acid residues at the N-terminus, being a highly potent antimicrobial molecule characterized by a spectrum of action with non-toxic effects in eukaryotes cell membranes (Kang et al., 2010).

Esculentin-1GN (ESC-1GN; GLFSKKGGKGGKSWIKGVFKGIKGIG-KEVGGDVIRTGIEIAACKIKGEC), from *Sylvirana guentheri* frog skin (Zeng et al., 2018), is a multifunctional peptide with antimicrobial, anti-inflammatory, lipopolysaccharide (LPS)-binding, antioxidant and antiviral activities against Influenza A (Zeng et al., 2018, 2020; Ye et al., 2020). Yang et al. (2021) found that ESC-1GN can suppress influenza virus H5N1 fusion activity by interacting with the subunit HA2. HA2 along with HA1 are subunits present on the HA surface glycoprotein, which play an important role in virus entry into host cells (Huang et al., 2013). ESC-1GN did not demonstrate activity against the subunit responsible for the virus-receptor interaction – HA1. However, it interfered with the fusion of the viral cell membrane, which is mediated by the HA2 subunit, driving to entry inhibition of the H5N1 virus into the host cell. Similar to Urumin (Holthausen et al., 2017) described,

Esculentin is one of the only AVPs with antiviral action against the Influenza A vírus.

5.5. Magainins

Magainins, also known as PGS (glycine serine peptide), are host defense peptides that were initially identified *Xenopus laevis* skin secretions (Zasloff, 1987; Giovannini et al., 1987). Magainins are also present in other species of the genus, such as *X. borealis, X. clivii, X. muelleri, X. petersii, X. amieti* and *X. andrei* (Conlon et al., 2012). In the *Xenopus* genus, there are some different Magainins that differ by small differences between their amino acid sequences (Xu and Lai, 2015). In this genus, we find Magainin 1, Magainin 2, Magainin-AM1, Magainin-AM2, Magainin-F3, Magainin-L1, Magainin-L1, Magainin-L1, Magainin-M1, Megainin-M2, Magainin-MW1, Maganin-P1, and Meganin-P2 (Ladram and Nicolas, 2016). These peptides are composed of 21 to 27 amino acids, being amphipathic, cationic, and α-helical (Sato and Feix, 2006).

Magainins have been potent antiviral agents (Chen et al., 1998; Zairi et al., 2009). Several Magainin variants had lysine-rich regions or many lysine residues in their structures, showing the best results in virus inhibition. Previous research has suggested that the cationic charges associated with amphipathic structures may allow these peptides to interact with anionic phospholipids in the viral envelopes, disrupting their structures by several unknown mechanisms (Dean et al., 2010). Egal et al. (1999) evaluated the activity of several synthetic Magainin against the Herpes Simplex-1 virus (HSV-1) and demonstrated that they could reduce viral amounts *in vitro* assays. This antiviral effect was higher when the HSV infection was pretreated with the peptides before inoculation into Vero cell monolayers, suggesting a direct virion effect (Egal et al., 1999). Both Magainin-1 and Magainin-2 exhibited an inhibitory action against Herpes Simplex-1 and 2 (HSV-1 and HSV-2) viruses at concentrations that are not toxic to epithelial cells (Albiol Matanic and Castilla, 2004). Thus, Magainin peptides are potential targets as biomedical agents for the treatment or prevention of viral infections.

5.6. Temporins

Temporins are anuran AMPs family (Conlon, 2006; Mangoni, 2006), with very short peptide sequences (8–17 amino acid residues) and are present in the *Ranidae* family (Wang, 2015). Temporins were first identified in the Asian frog *Rana erythraea* (Yashuhara et al., 1986) and European hybrid frog *Rana esculenta* (Simmaco et al., 1990), based on their hemolytic activity. These peptides are characterized by a weak cationic charge (ranging from +2 to +3) due to the presence of only one or two positively charged amino acids, such as lysine or arginine, in their sequence (Marcocci et al., 2018).

Temporin-SHa (SHa) (FLSGIVGMLGKLF) is a 13-amino acid peptide produced by the cutaneous granular glands of the North African frog *Pelophylax saharicus* (Ranidae; Abbassi et al., 2008) and has antiviral activity (Roy et al., 2019). It has a low net positive charge (+2), and its amphipathic helix structure allows interaction with the microbial cytoplasmic membrane, promoting pore formation and membrane disruption (Abbassi et al., 2008; Ladram and Nicolas, 2016). Roy et al. (2019) evaluated the capacity of SHa to inhibit herpes simplex virus 1 (HSV-1) replication during infections of primary cultures of human keratinocytes and observed that the antiviral action is primarily and directly on the viral particle, rather than indirectly, through an immunomodulatory mechanism. Nonetheless, the exact antiviral action mechanism has not yet been detailed.

Temporin-Tb (LLPIVGNLLKSLL), which has 50% homology with SHa, also has antiviral action against HSV-1 *in vitro* (Marcocci et al., 2018). Temporin-Tb can inhibit the initial stage of the multiplication cycle viral, inhibiting HSV-1 infection and preventing the spread of infected cells to neighboring uninfected cells (Marcocci et al., 2018).

Antivirals available for the treatment of HSV-1, such as acyclovir and derivatives, may lose their effectiveness in immunocompromised patients who require long-term treatment due develop resistance to these drugs (Piret and Bovin,2011). Thus, Temporins can be considered preventive antiherpetic agents or therapeutic agents, mainly for topical use.

5.7. Urumins

While most AMPs act by destabilizing membranes, peptides with specificity for cell surface molecules, although rare, have also been described. Urumin (IPLRGAFINGRWDSQCHRFSNG AIACA) is a peptide secreted by the Indian frog belonging to the Ranidae family, *Hydrophylax bahuvistara* (Vineeth Kumar et al., 2017). Holthausen et al. (2017) found that Urumin can inhibit the human influenza A virus. Through electrical stimulation, they collected animals' skin secretion and identified 32 AMPs. After molecular cloning, they synthesized these peptides to trace which one would have potent antiviral activity against the influenza A – H1N1 virus. They found that four peptides showed a greater than 50% reduction in viral load *in vitro*, and one of them showed no toxicity when incubated with human red blood cells (RBCs) at a concentration of up to 320 mM in PBS. This peptide, which they called Urumin, has 27 amino acids with a net positive charge and no known homology to any other AMP.

The action of Urumin is specific to hemagglutinin H1 and targets the hemagglutinin protein, a conserved region of the stem, where many specific antibodies are largely neutralized (HA; Pica and Palese, 2013; Krammer et al., 2015). The specifically bind between Urumin and hemagglutinin HA can cause a viral disruption, probably through a combination of conformational changes and electrostatic forces on the membrane. The antiviral effect of Urumin was specific for the influenza A virus since Urumin had no antiviral effect on any of the other viruses tested, which included HIV, SIV, HSV-II, hepatitis C, Ebola, Zika, and Dengue viruses (Holthausen et al., 2017). Thus, Urumin may be a useful antiviral therapy because it is effective against drug-resistant influenza H1 strains.

5.8. Phylogenetic analyses

Based on their peptide sequences, a phylogenetic analysis of all the AVPs cited above that demonstrated some action against human viruses was made. Further analyses showed two distinct clades (Fig. 2). The AVPs from *Ranidae* and *Hylidae* families were grouped in Clade I, while the AVPs from *Pelodryadidae*, *Phyllomedusidae*, and *Pipidae* were grouped in Clade II. There is a close taxonomic relationship among the families *Phyllomedusidae* and *Pelodryadidae*, which, together with *Hylidae*, create a monophyletic group in the anurans tree of life (Schulte et al., 2020) and could be a reason for this proximity among the AVPs. However, in this present analysis, the AVP from *Hylidae*, HS-1, seems to be distinct from those of *Pelodryadidae* and *Phyllomedusidae*, being grouped in Clade I with the AVPs of *Ranidae*.
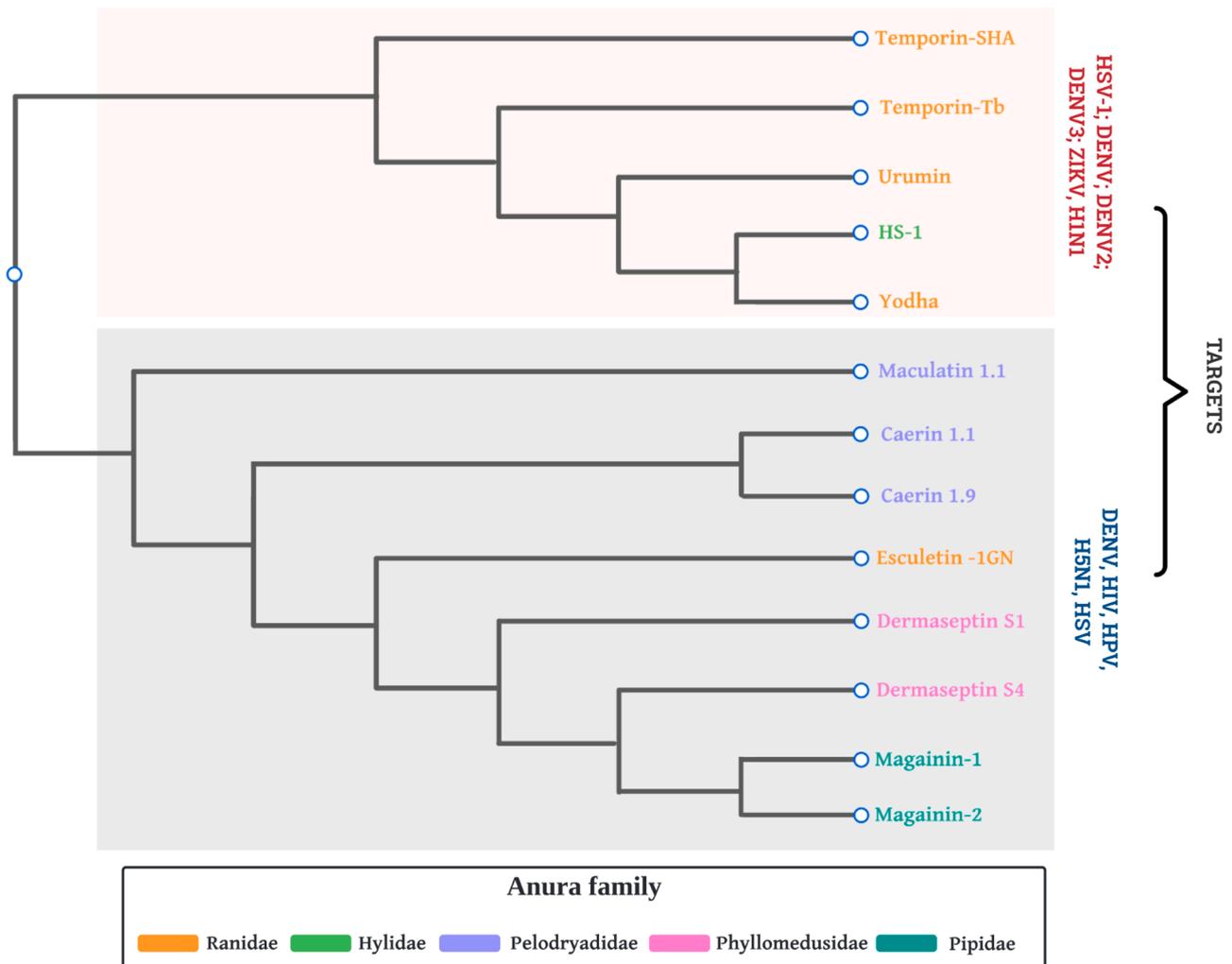

Fig. 2. Phylogenetic analysis showing the anurans skin peptides that had demonstrated antiviral action against human virus. Family indicators are expressed in colors.

Although the anuran families *Hylidae* and *Ranidae* belong to separated taxonomic clades (AmphibiaWeb, 2019; Schulte et al., 2020), the phylogenetic relationship among the AVPs may be related to their targets and mechanisms of action. The monophyletic group formed by HS-1 and Yodha, which have antiviral activity against Dengue virus, supports that hypothesis. Antiviral peptides effective against Dengue, Influenza, and Herpes simplex viruses were present in both clades. The Yodha peptide with antiviral activity against Zika virus was grouped in Clade I, while the AVPs effective against HPV and HIV were found only in Clade II. Therefore, these results sustain the hypothesis of a phylogenetic relationship based on target similarity. This highlight demonstrates how essential the development of studies about peptides from anurans' skin is.

6. Therapeutic potentials of AVPs against SARS-Cov-2

Currently, different types of research in different areas are being evaluated as potential treatments for COVID-19. Genome sequencing (Zhang and Holmes, 2020), testing with existing drugs such as Remdesivir (Beigel et al., 2020), Hydroxychloroquine, and Azithromycin (Gautret et al., 2020), reuse of possible molecules, and molecular fitting approach through bioinformatics (Hasan et al., 2020; Parvez et al., 2020) are some lines of research aimed to find therapeutic agents to treat or prevent SARS-CoV-2 infection. Peptide-based antimicrobial therapy has become a promising field in pharmaceutical research, and a large number of peptides are being tested for medicinal use (Fosgerau and Hoffmann, 2015). Therefore, AVPs give an interesting field to discover therapeutic tools against several infectious viral pathogens, including SARS-CoV-2.

Despite this, few studies have strong evidence that AVPs can provide alternative options as therapeutic agents against SARS-CoV-2. For example, a recent study suggested that a natural Lectin-like defensin-5 (HD5) peptide (ATCYCRTGRC ATRESLSGVCEISGRLYRLCCR) can successfully block angiotensin-converting enzyme 2 (ACE-2) receptors in the host, which has already been established that the ACE-2 receptor plays a vital role in the entry of SARS-CoV-2 into the human cellular system (Mahendran et al., 2020; Wang, 2020b). Another analysis of the probable efficacy of AVPs against coronaviruses was reported by Xia et al. (2019). From their study, they suggested that 'EK1' (SLDQINVTFLDLEYEMKKLEEAIKKLEESYIDLKEL) exhibited a significant level of cross-reactivity against all MERS-CoV and SARS-CoV (Xia et al., 2019). EK1 was found to be effective in inhibiting viral fusion entry (Xia et al., 2019; Mahendran et al., 2020, 2020). In addition, the viral envelope proteins of SARS-CoV, MERS-CoV, and influenza H5N1 viruses were disrupted by strong electrostatic affinity with an AVP called 'Mucroporin-M1' (LFRLIKSLIKRLVSAFK), which was designed as a peptide analog of the parent peptide Mucroporin (LFGLIPSLIGGLVSAFK) extracted the venom of the *Lychas mucronatus* scorpion (Dai et al., 2008; Li et al., 2011).

To choose the potential AVPs as therapeutic agents for Covid-19 infection, these peptides need to act in at least one of the life cycles stages of SARS-Cov-2. The life cycle of this virus includes (I) recognition of the viral membrane protein by the host receptor, (II) viral entry into the host cell, (III) release of viral RNA for replication, (IV) translation of polyproteins viral using host cell translation machinery, (V) synthesis of structural and non-structural viral proteins, and (VI) assembly and release of viral particles from the host cell through exocytosis (Bar-On et al., 2020; Wan et al., 2020). Furthermore, these potential antiviral AVPs must present specific characteristics that interfere with protein-protein interactions, block the substrate-binding site of main viral proteins, have a high intracellular half-life, and have a minimal marketable time (Kaspar and Reichert, 2013).

The AVPs described in anurans have many variable mechanisms of action, as the interaction with the viral cytoplasmic membrane; the promotion of membrane disruption through pore formation; the interaction directly in the viral particle through an immunomodulatory mechanism; the inhibition of the initial stage of the viral multiplication cycle; inhibition of infected cells; and the interruption of the infection through the disintegration of the virus before infecting the cell.

6.1. In silico studies to discover new potential AVPs

Since the beginning of the COVID-19 pandemic, applying computational studies has been extremely important to understanding the viral structure and phylogeny of SARS-CoV-2 (Hufsky et al., 2021). The relationship of SARS-CoV-2 with other bat coronaviruses was found through sequence analysis and structure prediction methods (Baruah et al., 2020; Li et al., 2020). Besides the morphologic and evolutionary aspects, *in silico* studies have shown to be the first step to initiating clinical and biological trials. This type of experiment usually uses machine learning to design and discover new molecules with antimicrobial activity through tests of the affinity between the subject and the target, as the peptide and the virus, respectively (Quintero-Gil et al., 2017; Liscano et al., 2020).

Some peptides and some drugs were recently identified through in silico experiments as potential candidates to treat COVID-19 disease (Wang et al., 2020; Çakır et al., 2021; Qin et al., 2021). Wang, 2020c was the first study to identify approved drugs that could be effective against the SARS-Cov-2, such as Lopinavir and Elbasvir. Through virtual docking, other studies found potential molecules from natural sources, such as green tea, red algae, and herbal plants (Joseph et al., 2021; Alam et al., 2021; Beirami et al., 2020). Currently, there are a lot of tools available to identify potential novel antiviral peptides, as well as their host-interaction, and the majority are free to use and available online, such as FIRM-AVP, VirusHostNet, CORDITE, and COVID-19 Docking Server (Chowdhury et al., 2020; Hufsky et al., 2021; Kong et al., 2020). Therefore, *in silico* studies could be a critical factor in discovering new therapeutic agents, such as AVPs, which can be crucial to new viral epidemics.

6.2. Magainins, Dermaseptins, and Caerins antiviral action against SARS CoV-2

There are few studies of natural molecular compounds that can inhibit the development of infections caused by SARS-CoV-2. For this reason, elucidating and describing possible natural inhibitors for this virus, such as antiviral peptides, are necessary for discovering alternative treatments. Due to the variety of biological activities and the targeting of the biochemical machinery of different pathogens or host cell structures, AVPs have obtained attention in the last year as a natural agent for combating and preventing Covid-19. *In silico* experiments using bioinformatics tools to perform protein-peptide docking and protein-protein docking simulations have gained even more attention as they can identify, evaluate and explore the molecular affinity and interaction of peptide molecules against SARS-CoV-2 (e.g. Jaiswal et al., 2020; Ling et al., 2020; Çakır et al., 2021). Following, we describe the studies that used Dermaseptins, Magainins, and Caerins peptides derived from anurans' skin, as potential antivirals against Covid-19.

6.2.1. Magainins and SARS-CoV-2

Recently, the antiviral mechanism of Magainin-1 and Magainin-2 against SARS-CoV-2 Mpro has been described, along with its effect in inhibiting the binding of ACE-2 receptors *in silico*. The SARS-CoV-2 virus has a principal protease that plays a key role in the viral replication and transcription called Mpro (Jin et al., 2020). As Mpro is unique in the virus and not found in host cells, this protease is a notable target for antiviral development against coronavirus infections (Yang et al., 2005). Newly, the resolution of the three-dimensional Mpro structure of SARS-CoV-2 allowed the investigation of potential inhibitors of viral replication (Zhang et al., 2020).

Fakih et al. (2020) used *in silico* approaches to indicate that the peptide molecules Magainin-1 and Magainin-2 have the potential to be

further developed as natural inhibitors of the SARS-CoV-2. First of all, they modeled the three-dimensional peptide sequence of Magainin-1 (GIGKFLHSAGKFGKAFVGEIMKS) and Magainin-2 (GIGKFLHSAKKFG-KAFVGEIMNS) derived from the frog skin (*Xenopus laevis*) using the PEP-FOLD 3.5 server (http://bioserv.rpbs.univ-paris-diderot.fr/PEP--FOLD/). The PEP-FOLD 3.5 is a server used to model peptide sequencing into three-dimensional conformation using the de novo method with amino acids between 5 and 50 (Maupetit et al., 2009; Thévenet et al., 2012; Chavan and Deobagkar, 2015; Lamiable et al., 2016). To anurans peptides, this method is highly indicated because it can obtain a peptide sequence without the protein database that overcomes the limitations of the methods that rely on peptide mass fingerprinting (PMF) databases. For this reason, can be used for non-sequenced organisms, antibodies, post-translational modification (PTM), and endogenous peptides (Bellows and Floudas, 2010). The macromolecule Mpro protease that was used in peptide-protein docking was obtained from Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 6LU7 (Jin et al., 2020) and the identification, evaluation, and exploration of the binding site area most responsible for the antiviral activity of the SARS-CoV-2 Mpro macromolecule were prepared using BIOVIA Discovery Studio 2016 (BIOVIA, 2016). Also, they made protein-protein docking simulations with Mpro and ACE-2 (Angiotensin Converting Enzyme-2 Receptor). The ACE-2 macromolecules were downloaded from the Protein Data Bank (http://www.rcsb.org/pdb) with PDB ID 2AJF (Li et al., 2005). The PatchDock was used to simulate protein docking of both peptide-protein complexes resulting from the protein-peptide docking methods (Sathya and Rajeswari, 2016; Aruleba et al., 2018) and the system's suitability was verified by visualization observations using BIOVIA Discovery Studio 2016 (BIOVIA, 2016).

The authors observed that all frog skin-derived antiviral peptides (*Xenopus laevis*) used in this study demonstrated affinity and interaction with the main protease (Mpro) of SARS-CoV-2. Magainin-2 has the best affinity for SARS-CoV-2 Mpro active site compared to Magainin-1. This result suggests that Magainin-2 can make stable bonds and interact in the macromolecule SARS-CoV-2 Mpro active site. Improved affinity with a strong and stable interaction of peptide molecules against SARS-CoV-2 Mpro may inhibit the entry into host cells and tissues due to coronavirus inability to contact ACE-2 receptors in the infection signaling process viral (Fakih et al., 2020).

In this sense, both Magainin-1 and Magainin-2 peptides can bind strongly and stably to the binding site area of the SARS-CoV-2 Mpro macromolecule. However, Magainin-2 has the best affinity and interaction with the active site and may also inhibit the formation of interactions with the surface of the ACE-2 receptor. Thus, the results indicate that Magainin-1 and Magainin-2 have the potential to be further developed as natural candidates to inhibitors of the SARS-CoV-2 Mpro macromolecules in the treatment of COVID-19 infectious diseases.

6.2.2. Dermaseptins and SARS-CoV-2

Dermaseptins, produced by Phyllomedusa genus frogs, also have elevated antiviral potential as seen above, and the peptides belonging to this group can be chosen for experimentation as an antiviral agent of SARS-CoV-2. During protein-peptide docking simulations, Dermaseptin-S9 showed a high affinity for the active site of SARS-CoV-2. This peptide was able to prevent the coupling of the spike protein SARS-CoV-2 to the ACE-2 receptor (Fakih, 2020) and can be used as an inhibitor of the virus spike glycoprotein (Satpathy, 2020).

Fakih (2020) performed an experiment through simulations of protein-peptide and protein-protein coupling *in silico*. For this, the spike protein macromolecule of SARS-CoV-2 was obtained through the Protein Data Bank (https://www.rcsb.org/) with PDB ID 6LZG (Wang et al., 2020) and the macromolecule of ACE-2 receptor downloaded also from the Protein Data Bank with PDB ID 2AJF (Li et al., 2005). The antiviral peptide molecules used were Dermaseptin-S4 (ALWMTLLKKVLKAAA-KAALNAVLVGANA) and Dermaseptin-S9 (GLRSKIWLWVLLMIW-QESNKFKKM), produced by frogs of the genus *Phyllomedusa* and

sequenced by PEP-FOLD 3.5 (http:// bioserv.rpbs.univ-paris-diderot. fr/PEP-FOLD/). The protein-peptide docking was performed using the HPEPDOCK algorithm (Huang and Zou, 2007, 2008; Yan et al., 2017; Zhou et al., 2018a, 2018b) and the analysis of the simulation results was carried out with Discovery Studio 2020 (Sharma, 2019; Sharma et al., 2019). The results of the protein-peptide docking simulation showed that Dermaseptin-S9 has the best affinity for the active binding site of the spike protein compared to Dermaseptin-S4, demonstrating that Dermaseptin-S9 is a promising inhibitor of the SARS-CoV-2 spike protein, due to strong binding and interactions in the active site area of the target macromolecule. When simulated protein-protein docking, both peptides prevent the coupling of the spike protein macromolecule SARS-CoV-2 to the surface of the ACE-2 receptor. However, the interactions formed by the Dermaseptin-S9 peptide were more numerous, stronger, and more stable compared to the Dermaseptin-S4 bonds.

In addition to this described study, Satpathy (2020), using *in silico* bioinformatics methods, tested different Dermaseptins to describe possible interactions between them and the spike protein of SARS-CoV-2. Through a search carried out in the database of antimicrobial peptides (at http://aps.unmc.edu/AP/main.php), three peptides Dermaseptin (originating from Phyllomedusa sauvagii) associated with the word antivirus were found: Dermaseptin -S1 (ALWKTMLKKLGT-MALHAGKAALGAAADTISQGTQ), Dermaseptin-S4 (ALWMTLLKKVL-KAAAKA ALNAVLVGANA), and Dermaseptin-S9 (GLRSKIWLWV LLMIWQESNKFKKM). Then, their three-dimensional structures were designed in PEP-FOLD 2.0 (https://mobyle.rpbs.univ-paris-diderot. fr/cgi-bin/portal.py#forms::PEP-FOLD). Protein-peptide docking tests were performed on the free online server (https://ncov.schanglab.org. cn) which can be used to discover therapeutic molecules against COVID-19 (Sukhwal and Sowdhamini, 2013). As a result, the overall interaction resulted in the maximum interaction that occurs between the Dermaseptin-S9 peptide and the A chain of the spike protein of the SARS-CoV-2 virus. Predicting that Dermaseptin-S9 could be the most useful antimicrobial peptide against the SARS-CoV-2 virus, as it shows a higher affinity for the SARS-CoV-2 spike glycoprotein compared to ACE2 and other non-microbial helical peptides. According to the study, Dermaseptin-S9 proves to be a promising candidate for studies to control infectious diseases such as that caused by SRAS-CoV-2.

6.2.3. Caerins and SARS-CoV-2

As Magainins and Dermaseptins, Caerins have been identified as possible antiviral therapeutic agents against SARS-CoV-2. Caerins 1.6 (GLFSVLGAVAKHVLPHVVPVIAEK) and 1.10 (GLLSVLGSVAKHV LPHVVPVIAEKL) have been shown to interact with virus Sgp proteins, decreasing the viral fusion capacity in the host cell (Liscano et al., 2020). A recent study tested, in silico, the protein-peptides dockings associated with the virus, focusing on the interactions between AMPs and SARS-CoV-2 target proteins, such as the Sgp protein and ACE2 host cell receptor (Liscano et al., 2020). The AMPs were pre-selected according to their physicochemical properties from the APD3 AMP database using a clustering strategy by integration of the K-Means method and algorithm elbow test with R-Project software (Li and Wu, 2012; Syakur et al., 2018). They used the MLRC method of NPS@: network protein sequence analysis (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_mlr.pl) (Guermeur et al., 1999; Combet et al., 2000) when the AMPs did not have available information for their secondary structures. Besides, the crystallographic coordinates for the structure of the SARS-CoV-2 S Sgp in the prefusion conformation, and the host cell receptor ACE2 was retrieved from the protein structure database RCSB Protein Data Bank, with PDB ID 6VYB (Walls et al., 2020) and 1R4L (Towler et al., 2004). The in silico structural AMPs modeling and validation used the I-TASSER platform and database RCSB PDB (Roy et al., 2010). The molecular models were built for each peptide with MODELLER 9.14 and the structures analyzed were visualized with PyMOL (https://pymol.org/2). The AMPs-target proteins docking was performed using Autodock vina software (Trott and Olson, 2010) and by CB-DOCK online tool (Liu et al., 2019). Finally, the

results obtained were analyzed by Discovery Studio Visualizer version 2020 (BIOVIA, 2020).

Among the clusters of AMPs found, one of them includes 15 peptides that belong to the *Hylidae* anuran family including AMPs from *Aureins, Caerins, Maculatins, and Uperins* families. Among these 15 peptides, ten were from the Caerin family, and Caerins 1.6 and 1.10 showed higher interactions with the Sgp virus protein. The residues VAL17, VAL18, and LYS24 of Caerin 1.6 interacted primarily with TYR756, ARG995, and THR998 residues of the Sgp virus. Meanwhile, Caerin 1.10 residues VAL5, PRO19, GLU23, and LEU25 interacted with HIS49, THR51, ASN969, and ARG995 Sgp residues. Hydrophobic interactions were more common in *Caerins* compared to control peptides EK1 and SARS-HR2P (which already have experimentally shown activity against SARS-CoV-2). ARG995 was the common Sgp residue for binding *Caerins*, and Caerin 1.10 had the strongest interaction with that residue, blocking the S2 subunit which plays an essential role in viral fusion and entry into the host cell through ACE2. So, as *Magainins* and *Dermaseptins, Caerins* are potentially effective peptides with antiviral properties for SARS-CoV-2.

7. Conclusions and perspectives

As a consequence of the effects of the Covid-19 pandemic, the search for new treatments and possible antiviral agents has been raised, being the anurans skin peptides, as discussed here, a great source of opportunity for the discovery of new molecules that could be used in the treatment or the production of new drugs and immunomodulation. *In silico* experiments showed that peptides from the Caerins, Dermaseptins, and Magainins families are potentially effective AMPs with antiviral properties against SARS-CoV-2. Most of these peptides with anti-SARS-CoV-2 action interact to prevent the virus from entering the cell. The Magainins could bind the site of the SARS-CoV-2 Mpro macromolecule inhibiting the entry into host cells due to the inability to contact the ACE-2 receptor. The Dermaseptins could prevent the coupling of the spike protein to ACE-2 receptor. Finally, Caerins could interact with virus Sgp proteins, decreasing the viral fusion capacity in the host cell via ACE-2. The use of computational tools was crucial to these findings; however, these AMPs still need experimental validation for their therapeutic efficacy to be proven and to become an alternative to conventional antiviral drugs. In addition, AVPs already described as potential therapeutics against other viral agents, such as those belonging to Brevenins, Maculatins, Esculentins, Temporins, and Urumins families, may also be targets of studies to verify their antiviral potential against SARS-CoV-2. The phylogenetic analyses performed in this study showed that these peptides with activity against SARS-Cov-2 are present in frogs from different families, which suggests that other anuran species could also produce peptides with antiviral activities. Furthermore, we hypothesized the antiviral action is in the interaction of the peptide with the virus in different manners, principal interacting direct to virus proteins or with the host cell receptors (ACE2) to inhibit the virus entry. Our study encourage the development of future studies to evaluate these interactions to validate that antiviral function and expand the possibilities of finding therapeutic agents against Covid-19.

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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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References

Abbassi, F., Oury, B., Blasco, T., Sereno, D., Bolbach, G., Nicolas, P., Hani, K., Amiche, M., Ladram, A., 2008. Isolation, characterization and molecular cloning of new temporins from the skin of the North African ranid *Pelophylax saharica*. Peptides Volume 29 (Issue 9), 1526–1533. https://doi.org/10.1016/j.peptides.2008.05.008. PagesISSN 0196-9781.

Agarwa, I.G., Gabrani, R., 2021. Antiviral peptides: identification and validation. Int. J. Pept. Res. Ther. 27 (1), 149–168. https://doi.org/10.1007/s10989-020-10072-0, 2021.

Ahmed, S.F., Ahmed, A.Q., Matthew, R.M., 2020. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses 12 (3), 254. https://doi.org/10.3390/v12030254.

Ahmed, A., Siman-Tov, G., Hall, G., Bhalla, N., Narayanan, A., 2019. Human antimicrobial peptides as therapeutics for viral infections. Viruses 11, 704. https://doi.org/10.3390/v11080704.

Albiol Matanic, C.C., Castilla, V., 2004. Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. Int. J. Antimicrob. Agents 23 (4), 382–389. https://doi.org/10.1016/j.ijantimicag.2003.07.022.

Alam, M.A., Parra-Saldivar, R., Bilal, M., Afroze, C.A., Ahmed, M.N., Iqbal, H.M.N., Xu, J., 2021. Algae-derived bioactive molecules for the potential treatment of SARS-CoV-2. Molecules, 26, 2134. https://doi.org/10.3390/molecules26082134.

Alexander, J.L., Thompson, Z., Cowan, J.A., 2018. Antimicrobial Metallopeptides. ACS Chem. Biol. 13 (6), 844–853. https://doi.org/10.1021/acschembio.7b00989.

Ali, M.F., Lips, K.R., Knoop, F.C., Fritzsch, B., Miller, C., Conlon, J.M., 2002. Antimicrobial peptides and protease inhibitors in the skin secretions of the crawfish frog, Rana areolata. Biochim. Biophys. Acta 1601, 55–63. https://doi.org/10.1016/S1570-9639(02)00432-6.

Ali, R., Rani, R., Kumar, S., Ashraf, G.M.D., Sheikh, I.A., 2013. New peptide based therapeutic approaches. editors Advances in Protein Chemistry. OMICS Group eBooks, Jeddah.

AmphibiaWeb, 2019. Family Phylogeny of Amphibia. University of California, Berkeley, CA, USA. Available online at: <https://amphibiaweb.org/> (accessed 28 March 2022).

Andersen, J.H., Jenssen, H., Gutteberg, T.J., 2003. Lactoferrin and lactoferricin inhibit Herpes simplex 1 and 2 infection and exhibit synergy when combined with acyclovir. Antivir. Res. 58, 209–215. https://doi.org/10.1016/S0166-3542(02)00214-0.

AmphibiaWeb, 2022. Available online at: www.amphibiaweb.org (accessed 23 February 2022).

APD. 2022. Antimicrobial peptide database. Last updated: Jan 6, 2022 | Copyright 2003-2022 Department of Pathology & Microbiology, UNMC, All Rights Reserved. Available online at: https://aps.unmc.edu/(accessed 26 February 2022).

Arouri, A., Dathe, M., Blume, A., 2009. Peptide induced demixing in PG/PE lipid mixtures: a mechanism for the specificity of antimicrobial peptides towards bacterial membranes? Biochim. Et Biophys. Acta Biomembr. 1788 (3), 650–659. https://doi.org/10.1016/j.bbamem.2008.11.022.

Aruleba, R.T., Adekiya, T.A., Oyinloye, B.E., Kappo, A.P., 2018. Structural studies of predicted ligand binding sites and molecular docking analysis of Slc2a4 as a therapeutic target for the treatment of cancer. Int. J. Mol. Sci. vol. 19 (2), 1–15. https://doi.org/10.3390/ijms19020386.

Badani, H., Garry, R.F., Wimley, W.C., 2014. Peptide entry inhibitors of enveloped viruses: the importance of interfacial hydrophobicity. Biochim. Et Biophys. Acta Biomembr. 1838, 2180–2197. https://doi.org/10.1016/j.bbamem.2014.04.015.

Barlow, P.G., Findlay, E.G., Currie, S.M., Davidson, D.J., 2014. Antiviral potential of cathelicidins. Future Microbiol. 9 (2014), 55–73. https://doi.org/10.2217/fmb.13.135.

Baruah, C., Devi, P., Sharma, D.K., 2020. Sequence analysis and structure prediction of SARS-CoV-2 accessory proteins 9b and ORF14: evolutionary analysis indicates close relatedness to bat coronavirus. Biomed. Res. Int. (2020), 7234961 https://doi.org/10.1155/2020/7234961.

Bar-On, Y.M., Flamholz, A., Phillips, R., Milo, R., 2020. SARS-CoV-2 (COVID-19) by the numbers. Elife 9 (2020). https://doi.org/10.7554/eLife.57309.

Basir, Y.J., Knoop, F.C., Dulka, J., Conlon, J.M., 2000. Multiple antimicrobial peptides and peptides related to brady-kinin and neuromedin N isolated from skin secretions of the pickerel frog, *Rana palustris*. Biochim. Biophys. Acta 1543, 95–105. https://doi.org/10.1016/S0167-4838(00)00191-6.

Beigel, J.H., Tomashek, K.M., Dodd, L.E., Mehta, A.K., et al., 2020. Lane Remdesivir for the treatment of covid-19 — final report. N. Engl. J. Med. (2020) https://doi.org/10.1056/NEJMoa2007764.

Beirami, A.D., Hatamabadi, D., Iranpanah, S., Rezaei, M., Ziai, S.A., 2020. In Silico identification of potentially effective herbal inhibitors of SARS-Cov-2 main protease by virtual screening method: potential anti-COVID-19 Molecules. Sch. Med. Stud. J. 2 (3), 2–6. https://doi.org/10.22037/smsj.v2i3.31997.

Belaid, A., Aouni, M., Khelifa, R., Trabelsi, A., Jemmali, M., Hani, K., 2002. *In vitro* antiviral activity of Dermaseptins against herpes simplex virus type 1. J. Med. Virol. 66 (2), 229–234. https://doi.org/10.1002/jmv.2134, 2002 Feb.

Bellows, M.L., Floudas, C.A., 2010. Computational methods for de novo protein design and its applications to the human immunodeficiency virus 1, purine nucleoside phosphorylase, ubiquitin specific protease 7, and histone demethylases. Curr. Drug Targets vol. 11 (3), 264–278. https://doi.org/10.2174/138945010790711914.

Bergaoui, I., Zairi, A., Tangy, F., Aoun, i M., Selmi, B., Hani, K., 2013. *In vitro* antiviral activity of dermaseptin S(4) and derivatives from amphibian skin against herpes simplex virus type 2. J. Med. Virol. 85 (2), 272–281. https://doi.org/10.1002/jmv.23450.

BIOVIA, 2016. Discovery studio modeling environment, Release 2018, San Diego: Dassault Systèmes.

BIOVIA. 2020. Discovery studio modeling environment, release 2020; Dassault Systèmes: San Diego, CA, USA.

Bowie, J.H., Separovic, F., Tyler, M.J., 2012. Host-defense peptides of Australian anurans. Part 2. Structure, activity, mechanism of action, and evolutionary significance. Peptides 37, 174. https://doi.org/10.1016/j.peptides.2012.06.017.

Çakır, B., Okuyan, B., Şener, G., Tunali-Akbay, T., 2021. Investigation of beta-lactoglobulin derived bioactive peptides against SARS-CoV-2 (COVID-19): in silico analysis. Eur. J. Pharmacol. 15 (891), 173781 https://doi.org/10.1016/j.ejphar.2020.173781.

Calhoun, D.M., Woodhams, D., Howard, C., LaFonte, B.E., Gregory, J.R., Johnson, P.T., 2016. Role of antimicrobial peptides in amphibian defence against trematode infection. Ecohealth 13, 383–391. https://doi.org/10.1007/s10393-016-1102-3.

Cardoso, J.L.M., de, S., Soares, M.J., dos, S., Leite, J.R.S.A., Malaquias, L.C.C., Coelho, L.F.L., 2013. Antiviral activity of dermaseptin 01 against Dengue virus type 2, Herpes simplex virus type 1 and Vaccinia virus. Sci. Med. 23 (1), 18–21. https://doi.org/10.15448/1980-6108.2013.1.12751 (Porto Alegre).

Chan, D.I., Prenner, E.J., Vogel, H.J., 2006. Tryptophan- and arginine-rich antimicrobial peptides: structures and mechanisms of action. Biochim. Biophys. Acta 1758, 1184–1202. https://doi.org/10.1016/j.bbamem.2006.04.006.

Chavan, S.G., Deobagkar, D.D., 2015. An in silico insight into novel therapeutic interaction of LTNF peptide-LT10 and design of structure based peptidomimetics for putative anti-diabetic activity. PLoS One vol. 10 (3), 1–20. https://doi.org/10.1371/journal.pone.0121860.

Chen, C.X., Hu, J., Zeng, P., Pan, F., Yaseen, M., Xu, H., Lu, J.R., 2014. Molecular mechanisms of anticancer action and cell selectivity of short alpha-helical peptides. Biomaterials 35 (5), 1552–1561. https://doi.org/10.1016/j.biomaterials.2013.10.082.

Chen, H.C., Brown, J.H., Morell, J.L., Huang, C.M., 1988. Synthetic Magainin analogues with improved antimicrobial activity. FEBS Lett. 236 (2), 462–466. https://doi.org/10.1016/0014-5793(88)80077-2, 1988 Aug 29.

Chen, Q., Wade, D., Kurosaka, K., Wang, Z.Y., Oppenheim, J.J., Yang, D., 2004. Temporin A and related frog antimicrobial peptides use formyl peptide receptor-like 1 as a receptor to chemoattract phagocytes. J. Immunol. 173 (4), 2652–2659. https://doi.org/10.4049/jimmunol.173.4.2652, 2004 Aug 15.

Chinchar, V.G., Bryan, L., Silphadaung, U., Noga, E., Wade, D., Rollins-Smith, L., 2004. Inactivation of viruses infecting ectothermic animals by amphibian and piscine antimicrobial peptides. Virology 323 (2), 268–275. https://doi.org/10.1016/j.virol.2004.02.029, 2004 Jun 1.

Chowdhury, A.S., Reehl, S.M., Kehn-Hall, K., et al., 2020. Better understanding and prediction of antiviral peptides through primary and secondary structure feature importance. Sci. Rep. 10, 19260. https://doi.org/10.1038/s41598-020-76161-8.

Cleaveland, S., Laurenson, M.K., Taylor, L.H., 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356, 991–999. https://doi.org/10.1098/rstb.2001.0889.

Colombo, B.M., Scalvenzi, T., Benlamara, S., Pollet, N., 2015. Microbiota and mucosal immunity in amphibians. Front. Immunol. 6, 111. https://doi.org/10.3389/fimmu.2015.00111.

Combet, C., Blanchet, C., Geourjon, C., Deléage, G., 2000. NPS@: network protein sequence analysis. Trends Biochem. Sci. 25 (2000), 147–150. https://doi.org/10.1016/s0968-0004(00)01540-6.

Conlon, J.M., et al., 2004. A family of brevinin-2 peptides with potent activity against Pseudomonas aeruginosa from the skin of the Hokkaido frog, Rana pirica. Regul. Pept. Volume 118 (Issue 3), 135–141. https://doi.org/10.1016/j.regpep.2003.12.003.

Conlon, et al., 2019. Peptidomic analysis in the discovery of therapeutically valuable peptides in amphibian skin secretions. Expert Review of Proteomics. https://doi.org/10.1080/14789450.2019.1693894.

Editor(s) Conlon, J.M., Kastin, A.J., 2006. The temporins - CHAPTER 46. Handbook of Biologically Active Peptides. Academic Press, pp. 305–309. https://doi.org/10.1016/B978-012369442-3/50049-0. PagesISBN 9780123694423.

Conlon, J.M., et al., 2014. Potential therapeutic applications of multifunctional host-defense peptides from frog skin as anti-cancer, anti-viral, immunomodulatory, and anti-diabetic agents. Peptides 7 (57), 67–77. https://doi.org/10.1016/j.peptides.2014.04.019.

Conlon, J.M. and Sonnevend, A. 2010. Antimicrobial peptides in frog skin secretions. In: Giuliani A., Rinaldi A. (eds) Antimicrobial Peptides. Methods in Molecular Biology (Methods and Protocols), vol. 618. Humana Press, Totowa, NJ. 10.1007/978-1-60761-594-1_1.

Conlon, J.M., 2011. Structural diversity and species distribution of host-defense peptides in frog skin secretions. Cell. Mol. Life Sci. 68 (2011), 2303–2315. https://doi.org/10.1007/s00018-011-0720-8.

Coutinho, R.M. et al., 2021. Model-based estimation of transmissibility and reinfection of SARS-CoV-2 P1 variant. Med Rxiv; Doi: 10.1101/2021.03.03.21252706.

Dai, C., et al., 2008. Mucroporin, the first cationic host defense peptide from the venom of lychas mucronatus. Antimicrob. Agents Chemother. 52, 3967–3972. https://doi.org/10.1128/AAC.00542-08.

Dean, R.E., O'Brien, L.M., Thwaite, J.E., Fox, M.A., Atkins, H., Ulaeto, D.O., 2010. A carpet-based mechanism for direct antimicrobial peptide activity against vaccinia virus membranes. Peptides vol. 31 (11), 1966–1972. https://doi.org/10.1016/j.peptides.2010.07.028.

Dockray, G.J., Hopkins, C.R., 1975. Caerulein secretion by dermal glands in *Xenopus laevis*. J. Cell Biol. https://doi.org/10.1083/jcb.64.3.724, 64r724-733.

Duellman, D.E., Trueb, L., 1986. Biology of Amphibians. McGraw-Hill, New York, NY.

Egal, M., et al., 1999. Antiviral effects of synthetic membrane-active peptides on herpes simplex virus, type 1. Int. J. Antimicrob. Agents 13 (1), 57–60. https://doi.org/10.1016/s0924-8579(99)00094-1.

Elkan, E., 1968. Mucopolysaccharides in the anuran defence against desiccation. J. Zool. 155 (1968), 19–53. https://doi.org/10.1111/j.1469-7998.1968.tb03028.x.

Epand, R.M., 2016. Host Defense Peptides and Their Potential as Therapeutic Agents, 1st ed. Springer International Publishing, p. 321. https://doi.org/10.1007/978-3-319-32949-9. pages.

Fakih, T.M., 2020. Dermaseptin-based antiviral peptides to prevent COVID-19 through in silico molecular docking studies against SARS-CoV-2 spike protein. Pharm. Sci. 7 (4), 8. https://doi.org/10.7454/psr.v7i4.1079.

Fakih, T.M., Dewi, M.L., Syahroni, E., 2020. Magainin as an antiviral peptide of SARS-CoV-2 main protease for potential inhibitor: an in silico approach. Biog. J. Ilm. Biol. vol. 8 (1), 89–95. https://doi.org/10.24252/bio.v8i1.11115 pISSN 2302-1616, eISSN 2580-2909June 200.

Falco, A., et al., 2007. Dual antiviral activity of human alpha-defensin-1 against viral haemorrhagic septicaemia rhabdovirus (VHSV): inactivation of virus particles and induction of a type I interferon-related response. Antivir. Res. 76, 111–123. https://doi.org/10.1016/j.antiviral.2007.06.006.

Farquhar, M.G., Palade, G.E., 1965. Cell junctions in amphibian skin. J. Cell Biol. 26 (1965), 263–291. https://doi.org/10.1083/jcb.26.1.263.

Fernebro, J., 2011. Fighting bacterial infections-future treatment options. Drug Resist. Updates 14 (2), 125–139. https://doi.org/10.1016/j.drup.2011.02.001.

Field, H.J., De Clercq, E., 2004. Antiviral drugs-a short history of their discovery and development. Microbiol. Today 31 (2004), 58–61.

Forni, G., Mantovani, A., 2021. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Differ. 28, 626–639. https://doi.org/10.1038/s41418-020-00720-9.

Fosgerau, K., Hoffmann, T., 2015. Peptide therapeutics: current status and future directions. Drug Discov. Today 20. https://doi.org/10.1016/j.drudis.2014.10.003, 122-12.

Fremont-Rahl, J.J., 2011. Mycobacterium liflandii outbreak in a research colony of *Xenopus* (Silurana) *tropicalis* frogs. Vet. Pathol. 48, 856–867. https://doi.org/10.1177/0300985810388520.

Gautret, P., Lagier, J.C., Parola, P., Hoang, V.T., Meddeb, L., Mailhe, M., et al., 2020. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int. J. Antimicrob. Agents 56, 105949. https://doi.org/10.1016/j.ijantimicag.2020.105949.

Gentilucci, L., De Marco, R., Cerisoli, L., 2010. Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. Curr. Pharm. Des. 16 (2010), 3185–3203. https://doi.org/10.2174/138161210793292555.

Giacometti, A., Cirioni, O., Ghiselli, R., Orlando, F., Silvestri, C., Renzone, G., Testa, I., Mocchegiani, F., Della Vittoria, A., Saba, V., Scaloni, A., Scalise, G., 2006. Distinctin improves the efficacies of glycopeptides and betalactams against staphylococcal biofilm in an experimental model of central venous catheter infection. J. Biomed. Mater. Res. A 81, 233–239. https://doi.org/10.1002/jbm.a.30982.

Giovannini, M.G., 1987. Biosynthesis and degradation of peptides derived from Xenopus laevis prohormones. Biochem. J. 243 (1), 113–120. https://doi.org/10.1042/bj2430113.

Guermeur, Y., Geourjon, C., Gallinari, P., Deléage, G., 1999. Improved performance in protein secondary structure prediction by inhomogeneous score combination. Bioinformatics 15 (1999), 413–421. https://doi.org/10.1093/bioinformatics/15.5.413.

Haney, E.F., Hancock, R.B., 2013. Peptide design for antimicrobial and immunomodulatory applications. Biopolymers 100, 572–583. https://doi.org/10.1002/bip.22250.

Hasan, M., Parvez, M.S.A., Azim, K.F., Imran, A.S., Raihan T., Gulshan, A., Muhit, S., Akhand, R.N., Uddin, M.B., Ahmed, S.S.U., 2020. Main protease inhibitors and drug surface hotspot for the treatment of COVID-19: drug repurposing and molecular docking approach. ChemRxiv (Preprint), 12118857. 10.1016/j.biopha.2021.111742.

Hoffman, C.W., Dent, J.N., 1977. Effects of neuro-transmitters upon the discharge of secretory product from the cutaneous glands of the red-spotted newt. J. Exp. Zool 202, 155–161. https://doi.org/10.1002/jez.1402020203.

Holthausen, D.J., et al., 2017. An amphibian host defense peptide is virucidal for human H1 hemagglutinin-bearing influenza viruses. Immunity Volume 46 (4), 587–595. https://doi.org/10.1016/j.immuni.2017.03.018. PagesISSN 1074-7613.

Hu, B., Guo, H., Zhou, P., Shi, Z.L., 2021. Characteristics of SARS-CoV-2 and COVID-19. Nat. Rev. Microbiol. 19 (3), 141–154. https://doi.org/10.1038/s41579-020-00459-7.

Huang, C., Wang, Y., Li, X., et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.

Huang, et al., 2013. Early steps of the conformational change of influenza virus hemagglutinin to a fusion active state—Stability and energetics of the hemagglutinin. Biochim. Biophys. Acta 1614, 3–13. https://doi.org/10.1016/s0005-2736(03)00158-5.

Huang, S.Y., Zou, X., 2007. Ensemble docking of multiple protein structures: considering protein structural variations in molecular docking. Proteins Struct. Funct. Genet. 66 (2), 399–421. https://doi.org/10.1002/prot.21214.

Huang, S.Y., Zou, X., 2008. An iterative knowledge based scoring function for protein-protein recognition. Proteins Struct. Funct. Genet. 72 (2), 557–579. https://doi.org/10.1002/prot.21949.

Hufsky, F., Lamkiewicz, K., Almeida, A., et al., 2021. Computational strategies to combat COVID-19: useful tools to accelerate SARS-CoV-2 and coronavirus research. Br. Bioinform. 22 (2), 642–663. https://doi.org/10.1093/bib/bbaa232.

Jaiswal, G., Kumar, V., 2020. In-silico design of a potential inhibitor of SARS-CoV-2 S protein. PLoS One 15 (10), e0240004. https://doi.org/10.1371/journal.pone.0240004.

Jenssen, H., et al., 2004. Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. Antivir. Res. 61, 101–109. https://doi.org/10.1016/j.antiviral.2003.09.001.

Jenssen, H., Hamill, P., Hancock, R.E.W., 2006. Peptide antimicrobial agents. Clin. Microbiol. Rev. 19, 491–511. https://doi.org/10.1128/CMR.00056-05.

Jin, Z., et al., 2020. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293. https://doi.org/10.1038/s41586-020-2223-y.

Joseph, et al., 2021. The use of Pseudotyped Coronaviruses for the Screening of Entry Inhibitors: Green Tea Extract Inhibits the Entry of SARS-CoV-1, MERSCoV, and SARS-CoV-2 by Blocking Receptor-spike Interaction. Current Pharmaceutical Biotechnology. https://doi.org/10.2174/1389201022666210810111716.

Kang, S.J., 2010. Solution structure of antimicrobial peptide esculentin-1c from skin secretion of Rana esculenta. Mol. Cells 30 (5), 435–441. https://doi.org/10.1007/s10059-010-0135-7.

Kaspar, A.A., Reichert, J.M., 2013. Future directions for peptide therapeutics development. Drug Discov. Today 807–817. https://doi.org/10.1016/j.drudis.2013.05.011.

Kim, J.B., et al., 2001. Antimicrobial peptides from the skin of the Japanese mountain brown frog, *Rana ornativentris*. J. Pept. Res. 58, 349–356. https://doi.org/10.1034/j.1399-3011.2001.00947.x.

Kong, R., Yang, G., Xue, R., Liu, M., Wang, F., Hu, J., Guo, X., Chang, S., 2020. COVID-19 docking server: a meta server for docking small molecules, peptides and antibodies against potential targets of COVID-19. Bioinformatics 36 (20), 5109–5111. https://doi.org/10.1093/bioinformatics/btaa645.

König, E., Bininda-Emonds, O.R.P., Shaw, C., 2015. The diversity and evolution of anuran skin peptides. Peptides 63, 96–117. https://doi.org/10.1016/j.peptides.2014.11.003. ISSN 0196-9781.

Koyama, T., Platt, D., Parida, L., 2020. Variant analysis of SARS-CoV-2 genomes. Bull. World Health Organ. 98 (7), 495–504. https://doi.org/10.2471/BLT.20.253591.

Krammer, F., Palese, P., Steel, J., 2015. Advances in universal influenza virus vaccine design and antibody mediated therapies based on conserved regions of the hemagglutinin. Curr. Top. Microbiol. Immunol. 386, 301–321. https://doi.org/10.1007/82_2014_408.

Ladram, A., Nicolas, P., 2016. Antimicrobial peptides from frog skin: biodiversity and therapeutic promises. Front. Biosci. 1 (21), 1341–1371. https://doi.org/10.2741/4461 (Landmark Ed).

Lamiable, A., Thévenet, P., Rey, J., Vavrusa, M., Derreumaux, P., Tufféry, P., 2016. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. Nucleic Acids Res. vol. 44 (1), 449–454. https://doi.org/10.1093/nar/gkw329.

Lee, E., et al., 2021a. Identification of SARS-CoV-2 nucleocapsid and spike T-cell epitopes for assessing T-cell immunity. J. Virol. 95 (6), e02002–e02020. https://doi.org/10.1128/JVI.02002-20.

Lee, S.H., Kim, E.H., O'neal, J.T., et al., 2021b. The amphibian peptide Yodha is virucidal for Zika and dengue viruses. Sci. Rep. 11, 602. https://doi.org/10.1038/s41598-020-80596-4.

Lequin, O., et al., 2006. Dermaseptin S9, an alpha-helical antimicrobial peptide with a hydrophobic core and cationic termini. Biochemistry 45, 468–480. https://doi.org/10.1021/bi051711i. -US.

Li, F., et al., 2005. Structural biology: structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science vol. 309 (5742), 1864–1868. https://doi.org/10.1126/science.1116480.

Li, Q., Grimm, M., Dai, W.T., Hou, M.C., Xiao, Z.X., Cao, Y., 2011. Virucidal activity of a scorpion venom peptide variant mucroporin-M1 against measles, SARS-CoV and influenza H5N1 viruses. Peptides 32, 1518–1525. https://doi.org/10.1016/j.peptides.2011.05.015.

Li, T., Liu, D., Yang, Y., et al., 2020. Phylogenetic supertree reveals detailed evolution of SARS-CoV-2. Sci. Rep. 10, 22366. https://doi.org/10.1038/s41598-020-79484-8.

Li, Y., Wu, H., 2012. A clustering method based on K-means algorithm. Phys. Procedia 25 (2012), 1104–1109. https://doi.org/10.1016/j.phpro.2012.03.206.

Lillywhite, H.B., 2006. Water relations of tetrapod integument. J. Exp. Biol. 209 (2006), 202–226. https://doi.org/10.1242/jeb.02007.

Ling, R., et al., 2020. In silico design of antiviral peptides targeting the spike protein of SARS-CoV-2. Peptides 130, 170328. https://doi.org/10.1016/j.peptides.2020.170328. VolumeISSN 0196-9781.

Liscano, Y., Oñate-Garzón, J., Ocampo-Ibáñez, I.D., 2020. In silico discovery of antimicrobial peptides as an alternative to control SARS-CoV-2. Molecules 25 (23), 5535. https://doi.org/10.3390/molecules25235535.

Liu, Y., Grimm, M., Dai, W.T., Hou, M.C., Xiao, Z.X., Cao, Y., 2019. CB-Dock: a web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacol. Sin. 41 (2019), 138–144. https://doi.org/10.1038/s41401-019-0228-6.

Lohner, K., Leber, R., 2016. Host Defense Peptides and Their Potential As Therapeutic Agents – Host Peptides Defense – Intracellular targets, 1st ed. Springer International Publishing, p. 321. https://doi.org/10.1007/978-3-319-32949-9. pages. Page 42.

Lorin, C., et al., 2005. The antimicrobial peptide dermaseptin S4 inhibits HIV-1 infectivity *in vitro*. Virology 334, 264–275. https://doi.org/10.1016/j.virol.2005.02.002.

Lou, Z., Sun, Y., Rao, Z., 2014. Current progress in antiviral strategies. Trends Pharmacol. Sci. 35, 86–102. https://doi.org/10.1016/j.tips.2013.11.006.

Lu, H., Stratton, C.W., Tang, Y.W., 2020. Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle. J. Med. Virol. 92 (4), 401–402. https://doi.org/10.1002/jmv.25678.

Maciel, N.M., Schwartz, C.A., Pires, O.R., Sebben, A., Castro, M.S., Sousa, M.V., et al., 2003. Composition of indolealkylamines of Bufo rubescens cutaneous secretionsc ompared to six other Brazilian bufonids with phylogenetic implications. Comp. Biochem. Physiol. B 134, 641–649. https://doi.org/10.1016/s1096-4959(03)00022-8.

Mahendran, A.S.K., et al., 2020. The potential of antiviral peptides as COVID-19 therapeutics. Front. Pharmacol. 11 https://doi.org/10.3389/fphar.2020.575444.

Mangoni, M., 2006. Temporins, anti-infective peptides with expanding properties. Cell. Mol. Life Sci. 63, 1060–1069. https://doi.org/10.1007/s00018-005-5536-y.

Mansour, S.C., Pena, O.M., Hancock, R.E., 2014. Host defense peptides: front-line immunomodulators. Trends Immunol. 35, 443–450. https://doi.org/10.1016/j.it.2014.07.004.

Marcocci, M.E., Amatore, D., Villa, S., et al., 2018. The amphibian antimicrobial peptide temporin B inhibits *in vitro* herpes simplex virus 1 infection. Antimicrob. Agents Chemother. 62 (5) https://doi.org/10.1128/AAC.02367-17, 2018e02367-17.

Matsuzaki, K., 1999. Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim. Biophys. Acta 1462, 1–10. https://doi.org/10.1016/S0005-2736(99)00197-2.

Maupetit, J., Derreumaux, P., Tuffery, P., 2009. PEP-FOLD: an online resource for de novo peptide structure prediction. Nucleic. Acids. Res. vol. 37, 498–503. https://doi.org/10.1093/nar/gkp323.

Mello, E.O., et al., 2011. Antifungal activity of PvD1 defensin involves plasma membrane permeabilization, inhibition of medium acidification, and induction of ROS in fungi cells. Curr. Microbiol. 62 (4), 1209–1217. https://doi.org/10.1007/s00284-010-9847-3.

Mettenleiter, T.C., 2001. Brief overview on cellular virus receptors. Virus Res. 82, 3–8. https://doi.org/10.1016/s0168-1702(01)00380-x.

Mishra, B., Reiling, S., Zarena, D., Wang, G., 2017. Host defense antimicrobial peptides as antibiotics: design and application strategies. Curr. Opin. Chem. Biol. 38, 87–96. https://doi.org/10.1016/j.cbpa.2017.03.014.

Migliolo, L., et al., 2012. Structural and functional characterization of a multifunctional alanine-rich peptide analogue from *Pleuronectes americanus*. PLoS One 7, e47047. https://doi.org/10.1371/journal.pone.0047047.

Mills, J.W., Prum, P.B., 1984. Morphology of the exocrine glands of the frog skin. Am. J. Anat. 171, 91–106. https://doi.org/10.1002/aja.1001710108.

Monteiro, J.M.C., Oliveira, M.D., Dias, R.S., Nacif-Marçal, L., Feio, R.N., Ferreira, S.O., Oliveira, L.L., Silva, C.C., Paula, So., 2018. The antimicrobial peptide HS-1 inhibits dengue virus infection. Virology 514 (2018), 79–87. https://doi.org/10.1016/j.virol.2017.11.009.

Mookherjee, N., Anderson, M.A., Haagsman, H.P., et al., 2020. Antimicrobial host defence peptides: functions and clinical potential. Nat. Rev. Drug Discov. 19 (2020), 311–332. https://doi.org/10.1038/s41573-019-0058-8.

Mor, A., Nicolas, P., 1994. The NH$_2$-terminal α-helical domain 1–18 of dermaseptin is responsible for antimicrobial activity. J. Biol. Chem. 269, 1934–1939.

Mor, A., Nguyen, V.H., Delfour, A., Migliore-Samour, D., Nicolas, P., 1991. Isolation, amino acid sequence, and synthesis of dermaseptin, a novel antimicrobial peptide of amphibian skin. Biochemistry 30 (36), 8824–8830. https://doi.org/10.1021/bi00100a014, 1991 Sep 10.

Morikawa, N., Hagiwara, K., Nakajima, T., 1992. Brevinin-1 and -2, unique antimicrobial peptides from the skin of the frog, *Rana brevipoda porsa*. Biochem. Biophys. Res. Commun. 189, 184–190. https://doi.org/10.1016/j.peptides.2005.06.024.

Morton, C.O., Santos, Dos, S.C, Coote, 2007. An amphibian-derived, cationic, alpha-helical antimicrobial peptide kills yeast by caspase-independent but AIF-dependent

programmed cell death. Mol. Microbiol. 65 (2), 494–507. https://doi.org/10.1111/j.1365-2958.2007.05801.x.

Mulder, K.C.L., et al., 2013. Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. Front. Microbiol. 4, 1–23. https://doi.org/10.3389/fmicb.2013.00321.

Nakashima, H., et al., 1992. Anti-human immunodeficiency virus activity of a novel synthetic peptide, T22 ([Tyr-5,12, Lys-7] polyphemusin II): a possible inhibitor of virus-cell fusion. Antimicrob. Agents Chemother. 36, 1249–1255. https://doi.org/10.1128/AAC.36.6.1249.

Ni, G., et al., 2018. Comparative proteomic study of the antiproliferative activity of frog host-defence peptide Caerin 1.9 and its additive effect with Caerin 1.1 on TC-1 cells transformed with HPV16 E6 and E7. Biomed. Res. Int. 2018, 7382351 https://doi.org/10.1155/2018/7382351.

Ni, G., et al., 2020. Host-defense peptides Caerin 1.1 and 1.9 stimulate TNF-alpha-dependent apoptotic signals in human cervical cancer HeLa cells. Front. Cell Dev. Biol. 8, 676. https://doi.org/10.3389/fcell.2020.00676.

Nicolas, P., El Amri, C., 2009. The dermaseptin superfamily: a gene-based combinatorial library of antimicrobial peptides. Biochim. Biophys. Acta 1788 (8), 1537–1550. https://doi.org/10.1016/j.bbamem.2008.09.006, 2009 Aug.

Nicolas, P., Ladram, A., Kastin, A., 2013. Dermaseptins. Handbook of Biologically Active Peptides, 110.1016/B978-0-12-385095-9.00050-6 Sect 5 Amphibian Skin Peptides.

Nicolas, P., Mor, A., 1995. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu. Rev. Microbiol. 49, 277–304. https://doi.org/10.1146/annurev.mi.49.100195.001425.

Noble, G.A., Noble, E.R., 1944. On the histology of frog skin glands. Trans. Am. Microsc. Soc. 63, 254–263. https://doi.org/10.2307/3223148.

Otvos, L., 2005. Antibacterial peptides and proteins with multiple cellular targets. J. Pept. Sci. 11 (11), 697–706. https://doi.org/10.1002/psc.698.

Pál, T., Abraham, B., Sonnevend, Á., Jumaa, P., Conlon, J.M., 2006. Brevinin-1BYa: a naturally occurring peptide from frog skin with broad-spectrum antibacterial and antifungal properties. Int. J. Antimicrob. Agents 27 (6), 525–529. https://doi.org/10.1016/j.ijantimicag.2006.01.010.

Pan, X., et al., 2019. Synthesized natural peptides from amphibian skin secretions increase the efficacy of a therapeutic vaccine by recruiting more T cells to the tumour site. BMC Complement. Altern. Med. 19, 163. https://doi.org/10.1186/s12906-019-2571-z.

Pantic, et al., 2017. The Potential of Frog Skin-Derived Peptides for Development into Therapeutically-Valuable Immunomodulatory Agents. Molecules. https://doi.org/10.3390/molecules22.

Parashar, U.D., Anderson, L.J., 2004. Severe acute respiratory syndrome: review and lessons from the 2003 outbreak. Int. J. Epidemiol. 33 (4), 628–634. https://doi.org/10.1093/ije/dyh198.

Park, J.M., Jung, J.E., Lee, B.J., 1994. Antimicrobial peptides from the skin of a Korean frog, *Rana rugosa*. Biochem. Biophys. Res. Commun. 205 (1), 948–954. https://doi.org/10.1006/bbrc.1994.2757.

Park, S., et al., 2001. Structural study of novel antimicrobial peptides, nigrocins, isolated from *Rana nigromaculata*. FEBS Lett. 507 (1), 95–100. https://doi.org/10.1016/s0014-5793(01)02956-8.

Park, J.E., Jung, S., Kim, A., et al., 2018. MERS transmission and risk factors: a systematic review. BMC Public Health 18, 574. https://doi.org/10.1186/s12889-018-5484-8.

Parvez, M.S.A., Karim, M.A., Hasan, M., et al., 2020. Prediction of potential inhibitors for RNA-dependent RNA polymerase of SARS-CoV-2 using comprehensive drug repurposing and molecular docking approach. Int. J. Biol. Macromol. 163, 1787–1797. https://doi.org/10.1016/j.ijbiomac.2020.09.098.

Peschel, A., Sahl, H.G., 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat. Rev. Microbiol. 4, 529–536. https://doi.org/10.1038/nrmicro1441.

Pica, N., Palese, P., 2013. Toward a universal influenza virus vaccine: prospects and challenges. Annu. Rev. Med. 64, 189–202. https://doi.org/10.1146/annurev-med-120611-145115.

Piret, G., Boivin, G., 2011. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. Antimicrob. Agents Chemother. 55, 459–472. https://doi.org/10.1128/AAC.00615-10.

Pukala, T.L., Brinkworth, C.S., Carver, J.A., Bowie, J.H., 2004. Investigating the importance of the flexible hinge in Caerin 1.1: solution structures and activity of two synthetically modified Caerin peptides. Biochemistry 43 (4), 937–944. https://doi.org/10.1021/bi035760b.

Qin, J., Guo, C., Yang, L., Liang, X., Jiao, A., Lai, K.P., Yang, B., 2021. Bioinformatics and in-silico findings reveal medical features and phamacological targets of biochanin A against colorectal cancer and COVID-19. Bioengineered 12 (2), 12461–12469. https://doi.org/10.1080/21655979.2021.2005876.

Quintero-Gil, C., Parra-Suescun, J., Lopez-Herrera, A., Orduz, S., 2017. In-silico design and molecular docking evaluation of peptides derivates from bacteriocins and porcine beta defensin-2 as inhibitors of Hepatitis E virus capsid protein. VirusDis 28 (3), 281–288. https://doi.org/10.1007/s13337-017-0383-7.

Reddy, K.V.R., Yedery, R.D., Aranha, C., 2004. Antimicrobial peptides: premises and promises. Int. J. Antimicrob. Agents 24 (6), 536–547. https://doi.org/10.1016/j.ijantimicag.2004.09.005.

Rinaldi, A.C., 2002. Antimicrobial peptides from amphibian skin: an expanding scenario. Curr. Opin. Chem. Biol. 6, 799–804. https://doi.org/10.1016/s1367-5931(02)00401-5.

Robert, J., Ohta, Y., 2009. Comparative and developmental study of the immune system in Xenopus. Dev. Dyn. 238 (6), 1249–1270. https://doi.org/10.1002/dvdy.21891.

Roy, A., Kucukural, A., Zhang, Y., 2010. I-TASSER: a unified platform for automated protein structure and function prediction. Nat. Protoc. 5 (2010), 725–738. https://doi.org/10.1038/nprot.2010.5.

Roy, M., Lebeau, L., Chessa, C., et al., 2019. Comparison of anti-viral activity of frog skin anti-microbial peptides temporin-sha and [K³] SHa to LL-37 and Temporin-Tb against herpes simplex virus type 1. Viruses 11 (1), 77. https://doi.org/10.3390/v11010077.

Rozek, T., et al., 1998. The maculatin peptides from the skin glands of the tree frog Litoria genimaculata: a comparison of the structures and antibacterial activities of maculatin 1.1 and Caerin 1.1. J. Pept. Sci. (2), 111–115. https://doi.org/10.1002/(sici)1099-1387(199804)4:2<111::aid-psc134>3.0.co;2-8.

Sato, H., Feix, J.B., 2006. Peptide-membrane interactions and mechanisms of membrane destruction by amphipathic alpha-helical antimicrobial peptides. Biochim. Biophys. Acta 1758 (9), 1245–1256. https://doi.org/10.1016/j.bbamem.2006.02.021.

Sathya, D., Rajeswari, V.D., 2016. In Silico docking analysis of bioactive compounds from Chinese medicine Jinqi Jiangtang Tablet (JQJTT) using Patch Dock. J. Chem. Pharm. Res. 5 (8), 15–21.

Satpathy, R., 2020. In silico prediction of anti–SARS-CoV-2 effect of dermaseptin peptides from amphibian origin: prediction of anti–SARS-CoV-2 effect of the dermaseptin peptides. Trends Pept. Protein Sci. 5, 1–9. https://doi.org/10.22037/tpps.v5i.32560.

Savoia, D., Donalisio, M., Civra, A., Salvadori, S., Guerrini, R., 2010. *In vitro* activity of dermaseptin S1 derivatives against genital pathogens. APMIS 118 (9), 674–680. https://doi.org/10.1111/j.1600-0463.2010.02637.x.

Syakur, M.A., Khotimah, B.K., Rochman, E.M.S., Satoto, B.D., 2018. Integration K-means clustering method and elbow method for identification of the best customer profile cluster. IOP Conf. Ser. Mater. Sci. Eng. 336, 12017.

Sharma, A., Pal, S., Panwar, A., Kumar, S., Kumar, A., 2021. In-silico immunoinformatic analysis of SARS-CoV-2 virus for the development of putative vaccine construct. Immunobiology 226 (5), 152134. https://doi.org/10.1016/j.imbio.2021.152134.

Sharma, S., 2019. Molecular Dynamics Simulation of Nanocomposites using BIOVIA Materials Studio, Lammps and Gromacs. In Sharma, S. (Ed.). Elsevier. https://doi.org/10.1016/C2017-0-04396-3.

Sharma, S., Sharma, S., et al., 2019. Overview of BIOVIA materials studio, LAMMPS, and GROMACS. Molecular Dynamics Simulation of Nanocomposites using BIOVIA Materials Studio, Lammps and Gromacs. Elsevier. https://doi.org/10.1016/C2017-0-04396-3.

Shartouny, J.R., Jacob, J., 2019. Mining the tree of life: host defense peptides as antiviral therapeutics. Semin. Cell Dev. Biol. 88, 147–155. https://doi.org/10.1016/j.semcdb.2018.03.001.

Schulte, L.M., Ringler, E., Rojas, B., Stynoski, J.L., 2020. Developments in amphibian parental care research: history, present advances, and future perspectives. Herpetol. Monogr. 34, 71–97. https://doi.org/10.1655/HERPMONOGRAPHS-D-19-00002.1.

Silva, O.N., Porto, W.F., Migliolo, L., et al., 2012. Cn-AMP1: a new promiscuous peptide with potential for microbial infections treatment. Biopolymers 98 (4), 322–331. https://doi.org/10.1002/bip.22071.

Simmaco, M., De Biase, D., Severini, C., et al., 1990. Purification and characterization of bioactive peptides from skin extracts of *Rana esculenta*. Biochim. Biophys. Acta 1033 (3), 318–323. https://doi.org/10.1016/0304-4165(90)90140-r.

Simmaco, M., Mignogna, G., Barra, D., Bossa, F., 1993. Novel antimicrobial peptides from skin secretion of the European frog *Rana esculenta*. FEBS Lett. 324 (2), 159–161. https://doi.org/10.1016/0014-5793(93)81384-c.

Simmaco, M., Mignogna, G., Barra, D., Bossa, F., 1994. Antimicrobial peptides from skin secretions of *Rana esculenta*. Molecular cloning of cDNAs encoding esculetin and brevinins and isolation of new active peptides. J. Biol. Chem. 269 (16), 11956–11961.

Simmaco, M., Mignogna, G., Barra, D., 1998. Antimicrobial peptides from amphibian skin: what do they tell us? Biopolymers 47 (6), 435–450. https://doi.org/10.1002/(SICI)1097-0282(1998)47:6<435::AID-BIP3>3.0.CO;2-8.

Stone, D.J.M, et al., 1992. ChemInform Abstract: Peptides from Australian Frogs. Structures of the Caerins and Caeridin 1 from *Litoria splendida*. ChemInform. https://doi.org/10.1002/chin.199312234.

Sukhwal, A., Sowdhamini, R., 2013. Oligomerization status and evolutionary conservation of interfaces of protein structural domain superfamilies. Mol. Biosyst. 9 (7), 1652–1661. https://doi.org/10.1039/c3mb25484d.

Suzuki, M., Hasegawa, T., Ogushi, Y., Tanaka, S., 2007. Amphibian aquaporins and adaptation to terrestrial environments: a review. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 148, 72–81. https://doi.org/10.1016/j.cbpa.2006.12.021.

Tamamura, H., Xu, Y., Hattori, T., et al., 1998. A low-molecular-weight inhibitor against the chemokine receptor CXCR4: a strong anti-HIV peptide T140. Biochem. Biophys. Res. Commun. 253, 877–882. https://doi.org/10.1006/bbrc.1998.9871.

Taylor, S., Massiah, A., Lomonossoff, G., et al., 1994. Correlation between the activities of five ribosome-inactivating proteins in depurination of tobacco ribosomes and inhibition of tobacco mosaic virus infection. Plant J. 5 (6), 827–835. https://doi.org/10.1046/j.1365-313X.1994.5060827.x.

Teixeira, L.D., Silva, O.N., Migliolo, L., et al., 2013. *In vivo* antimicrobial evaluation of an alanine-rich peptide derived from *Pleuronectes americanus*. Peptides 42, 144–148. https://doi.org/10.1016/j.peptides.2013.02.001.

Thévenet, P., Shen, Y., Maupetit, J., Guyon, F., Derreumaux, P., Tufféry, P., 2012. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. Nucleic Acids Res. 40, 288–293. https://doi.org/10.1093/nar/gks419.

Torcato, I.M., Huang, Y.H., Franquelim, H.G., et al., 2013a. Design and characterization of novel antimicrobial peptides, R-BP100 and RW-BP100, with activity against gram-negative and gram-positive bacteria. Biochim. Et Biophys. Acta Biomembr. 1828 (3), 944–955. https://doi.org/10.1016/j.bbamem.2012.12.002.

Torcato, I.M., Huang, Y.H., Franquelim, H.G., et al., 2013b. The antimicrobial activity of Sub3 is dependent on membrane binding and cell-penetrating ability. ChemBioChem 14 (15), 2013–2022. https://doi.org/10.1002/cbic.201300274.

Towler, P., Staker, B., Prasad, S.G., Menon, S., Tang, et al., 2004. ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J. Biol. Chem. 279 (17), 17996–18007. https://doi.org/10.1074/jbc.M311191200.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461. https://doi.org/10.1002/jcc.21334.

V'kovski, P., Kratzel, A., Steiner, S., et al., 2021. Coronavirus biology and replication: implications for SARS-CoV-2. Nat. Rev. Microbiol. 19 (3), 155–170. https://doi.org/10.1038/s41579-020-00468-6.

VanCompernolle, S., Smith, P.B., Bowie, J.H., et al., 2015. Inhibition of HIV infection by Caerin 1 antimicrobial peptides. Peptides 71, 296–303. https://doi.org/10.1016/j.peptides.2015.05.004.

VanCompernolle, S.E., Taylor, R.J., Oswald-Richter, K., et al., 2005. Antimicrobial peptides from amphibian skin potently inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. J. Virol. 79 (18), 11598–11606. https://doi.org/10.1128/JVI.79.18.11598-11606.2005.

Vilas Boas, L.C.P., Campos, M.L., Berlanda, R.L.A., et al., 2019. Antiviral peptides as promising therapeutic drugs. Cell. Mol. Life Sci. 76 (18), 3525–3542. https://doi.org/10.1007/s00018-019-03138-w.

Vineeth Kumar, T.P.V.K., Asha, R., Shyla, G., Georgia, S., 2017. Identification and characterization of novel host defense peptides from the skin secretion of the fungoid frog, *Hydrophylax bahuvistara* (Anura: ranidae). Chem. Biol. Drug Des. 92 (2), 1409–1418. https://doi.org/10.1111/cbdd.12937.

Wachinger, M., Kleinschmidt, A., Winder, D., et al., 1998. Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. J. Gen. Virol. 79 (4), 731–740. https://doi.org/10.1099/0022-1317-79-4-731.

Walls, A.C., Park, Y.J., Tortorici, M.A., et al., 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181 (1), 281–292. https://doi.org/10.1016/j.cell.2020.02.058 e6.

Wan, Y., Shang, J., Graham, R., Baric, R.S., Li, F., 2020. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J. Virol. 94 (7) https://doi.org/10.1128/JVI.00127-20 e00127-20.

Wang, C., Wang, S., Li, D., Zhao, X., et al., 2020. Lectin-like intestinal defensin inhibits 2019-nCoV spike binding to ACE2. bioRxiv. 10.1101/2020.03.29.013490.

Wang, G., 2012. Natural antimicrobial peptides as promising anti-HIV candidates. Curr. Top. Pept. Protein Res. 13, 93–110.

Wang, G., 2015. Improved methods for classification, prediction, and design of antimicrobial peptides. Methods Mol. Biol. 1268, 43–66. https://doi.org/10.1007/978-1-4939-2285-7_3.

Wang, Y., Zhang, Y., Lee, W.H., Yang, X., Zhang, Y., 2016. Novel peptides from skins of amphibians showed broad-spectrum antimicrobial activities. Chem. Biol. Drug Des. 87 (3), 419–424. https://doi.org/10.1111/cbdd.12672.

Wang, G., 2020a. Bioinformatic analysis of 1000 amphibian antimicrobial peptides uncovers multiple length-dependent correlations for peptide design and prediction. Antibiotics 9 (8), 491. https://doi.org/10.3390/antibiotics9080491 (Basel).

Wang, Junmei, 2020c. Fast Identification of Possible Drug Treatment of Coronavirus Disease-19 (COVID-19) through Computational Drug Repurposing Study. J. Chem. Inf. Model. https://doi.org/10.1021/acs.jcim.0c00179.

Wang, G., Epand, R.M., 2016. Host defense peptides and their potential as therapeutic agents. Structural Analysis of Amphibian, Insect, and Plant Host Defense Peptides Inspires the Design of Novel Therapeutic Molecules 229–251. https://doi.org/10.1007/978-3-319-32949-9_9. Chapter 9, Pages.

Wang, et al., 2017. Large-Scale Analysis of Antimicrobial Activities in Relation to Amphipathicity and Charge Reveals Novel Characterization of Antimicrobial Peptides. Molecules. https://doi.org/10.3390/molecules22112037.

Wang, Q, 2020b. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell. https://doi.org/10.1016/j.cell.2020.03.045.

Wells, K.D., 2007. The Ecology and Behaviour of Amphibians. Chicago: The University of Chicago Press.

WHO, 2022. Coronavirus (COVID-19) Dashboard,. WHO https://covid19.who.int. Accessed 28 March 2022.

Wong, H., Bowie, J.H., Carver, J.A., 1997. The solution structure and activity of Caerin 1.1, an antimicrobial peptide from the Australian green tree frog, *Litoria splendida*. Eur. J. Biochem. 247, 545–557. https://doi.org/10.1111/j.1432-1033.1997.00545.x.

Xia, S., Yan, L., Xu, W., et al., 2019. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. Sci. Adv. 5, 4. https://doi.org/10.1126/sciadv.aav4580.

Xia, S., Liu, M., Wang, C., Xu, W., et al., 2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res. 30, 343–355. https://doi.org/10.1038/s41422-020-0305-x.

Xu, X., Lai, R., 2015. The chemistry and biological activities of peptides from amphibian skin secretions. Chem. Rev. 115 (4), 1760–1846. https://doi.org/10.1021/cr4006704.

Yan, Y., Zhang, D., Huang, S.Y., 2017. Efficient conformational ensemble generation of protein-bound peptides. J. Cheminform. 9 (1), 59. https://doi.org/10.1186/s13321-017-0246-7.

Yang, H., Xie, W., Xue, X., Yang, K., et al., 2005. Design of wide-spectrum inhibitors targeting coronavirus main proteases. PLoS Biol. 3 (10), e324. https://doi.org/10.1371/journal.pbio.0030324.

Yang, J., Zhang, B., Huang, Y., et al., 2021. Antiviral activity and mechanism of ESC-1GN from skin secretion of *Hylarana guentheri* against influenza A virus. J. Biochem. 169 (6), 757–765. https://doi.org/10.1093/jb/mvab019.

Yashuhara, T., Kiso, Y., et al., 1986. Isolation and sequential analysis of peptides in *Rama erythraea* skin. Peptide Chemistry. Protein Research Foundation, Osaka, pp. 363–368.

Yasin, B., Pang, M., Turner, J.S., Cho, Y., et al., 2000. Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides. Eur. J. Clin. Microbiol. Infect. Dis. 19 (3), 187–194. https://doi.org/10.1007/s100960050457.

Ye, T., Wu, J., Xu, Z., Chai, J., et al., 2020. Esc-1GN shows therapeutic potentials for acne vulgaris and inflammatory pain. J. Pept. Sci. 26, e3269. https://doi.org/10.1002/psc.3269.

Yokoyama, H., Kudo, N., Todate, M., et al., 2018. Skin regeneration of amphibians: a novel model for skin regeneration as adults. Dev. Growth Differ. 60, 316–325. https://doi.org/10.1111/dgd.12544.

Zairi, A., Tangy, F., Bouassida, K., Hani, K., 2009. Dermaseptins and Magainins: antimicrobial peptides from frogs' skin-new sources for a promising spermicides microbicides-a mini review. J. Biomed. Biotechnol., 452567 https://doi.org/10.1155/2009/452567.

Zasloff, M., 1987. Magainins, a class of antimicrobial peptides from Xenopus skin –isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. U. S. A. 84 (15), 5449–5453. https://doi.org/10.1073/pnas.84.15.5449.

Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature 415 (6870), 389–395. https://doi.org/10.1038/415389a.

Zeng, B., Chai, J., Deng, Z., et al., 2018. Functional characterization of a novel lipopolysaccharide-binding antimicrobial and anti-inflammatory peptide *in vitro* and *in vivo*. J. Med. Chem. 61 (23), 10709–10723. https://doi.org/10.1021/acs.jmedchem.8b01358.

Zeng, Q., Zeng, B., Chai, J., et al., 2020. Antioxidant properties and neuroprotective effects of Ecs-1GN through the regulation of MAPK and AKT signaling. Life Sci. 254, 117753 https://doi.org/10.1016/j.lfs.2020.117753.

Zhang, L., Lin, D., Sun, X., et al., 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 368 (6489), 409–412. https://doi.org/10.1126/science.abb3405.

Zhang, Y.Z., Holmes, E.C., 2020. A genomic perspective on the origin and emergence of SARS-CoV-2. Cell 181, 223–227. https://doi.org/10.1016/j.cell.2020.03.035.

Zhou, P., Jin, B., Li, H., Huang, S.Y., 2018a. HPEPDOCK: a web server for blind peptide-protein docking based on a hierarchical algorithm. Nucleic Acids Res. 46, 443–450. https://doi.org/10.1093/nar/gky357.

Zhou, P., Li, B., Yan, Y., et al., 2018b. Hierarchical flexible peptide docking by conformer generation and ensemble docking of peptides. J. Chem. Inf. Model. 58 (6), 1292–1302. https://doi.org/10.1021/acs.jcim.8b00142.

Zhu, N., Zhang, D., Wang, W., et al., 2020. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382 (8), 727–733.