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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 promising 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 infections. 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 Brevininns, Maculatins, Esculetins, 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; Elkam et al., 1968; Lillywhitte 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 herpex simplex virus type 1 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 develop 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 Ceain, 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 arginine 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% triluuroethanol–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 degranulants/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),
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3. AMPs in amphibians

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 a chemotaxis of inflammatory cells towards the site exposed to the pathogen (Chen et al., 2004). The microbicidal 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, Esculetins, Temporins, Caerins, Ranalexins, Ranauterins, Palustrins, Tigerinins, Japonicins, Maculatins and Nigricins (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 and Brevinins, several families of peptides found in some frog species, including species from the Hylidae and Ranidae families (Nicolas and El Amri, 2009). Similar to Magainins, 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 amphipathic-derived cationic peptides that interacted with DNA in vitro (Lochner 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 (VanCompernelle 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 (Misra 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 (Albiol-Matanic and Castillo, 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 (Badami 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 CCR4 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 (Miglioli 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 DNA 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.

| Anuran AVP   | Peptide Family | Frog Species | Sequence | Virus       | Reference                  |
|--------------|----------------|--------------|----------|-------------|---------------------------|
| Caerin 1.1   | Caerin         | Litotia splendidida (Pelodyridae) | GLSGLVGLAVHHPVAVIAEHL | HPV and HIV | Stone et al., 1992        |
| Caerin 1.9   | Caerin         | Litotia caerulea (Pelodyridae)   | GLFGLGIAKIHHPVAVIAEKL | HIV and HSV | VanCompernolle et al., 2005 |
| Dermaseptin S4| Dermaseptin    | Liotrechus chloris (Pelodyridae) | ALWMLKKLKVIAAKAALNAVŁGANA | HIV and HSV | Ni et al., 2018           |
| Dermaseptin S1| Dermaseptin    | Phylomedusa (Phylopomidae)       | ALWKLMLKGLTMALHGAGGAIUDTSQCTQ | HSV and DENV | Lorin et al., 2005        |
| Esculentin-1GN| Esculentin     | Syplorena guentheri (Ranidae)    | GLFSKGGGKKGKSWGKVFVIGKIGKEVGGD | HSNI         | Bergou et al., 2013        |
| HS-1         | Unclassified   | Hylidae      | H1-FLPPLPSVIALKLSLFGQ-OH | DENV 2 and DENV 3 | Yang et al., 2021         |
| Maculatin 1.1| Maculatin      | Litoria gencincipalta (Pelodyridae) | GLFGLVARKIAHHPVPAIAEHF | HPV and HIV | Ni et al., 2018           |
| Magainin-2   | Magainin       | Xenopus (Pipidae)                | GIGRFILASAGKFGKAFTGEIMK | HSV         | VanCompernolle et al., 2005 |
| Temporin-SHa | Temporin       | Perophylax saharicus (Ranidae)   | GILFGFIKSKFPGKAFVEAIMNS | HSV-1       | Yu et al., 2019           |
| Temporin-Tb  | Temporin       | Rana temporaria (Ranidae)        | LLLPVPGLMKSL | HSV-1      | Marcocci et al., 2018     |
| Urumin       | Urumin         | Hydrophilax bahivistora (Ranidae) | IPLRAGINGIRGDSQCHRFSNGAACA | H1N1      | Holthausen et al., 2017   |
| Yodha        | Yodha          | Indosylvana aurantica (Ranidae)  | SMLLIFFGTSLSLQDDQDQRC | 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
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 (SMLLLFLGTISLSLCQDDQERC) 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 (GILLSVLSVAKHLPHVVPVIAEHL) and Caerin 1.9 (GLFGVLGSIAKHLPHVVPVIAEKIL) were originally isolated from Australian tree frog skin of the genus Litoria, 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 (GLFGVVLKVAHVVPVIAEHF), 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 Ladrán, 2013). These 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 asparatate). 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, Bergau 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 endomtrial cells (HEC-1) and HIV-1 transcytosis through a compact HEC-1 monolayer. Dermaseptin S1 exhibited antiviral activity against herpes simplex virus type 1 (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 (Ladrám 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. Esculentin

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;巴斯尔 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 potant antimicrobial molecule characterized by a spectrum of action with non-toxic effects in eukaryotes cell membranes (Kang et al., 2010).

Esculentin-1GN (ESC-1GN; GLFSKGGKGGWIKGFVFKGIG-KEVGGDIVRGHEIAACKIKGEC), 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 Uromin (Holthausen et al., 2017) described, Esculentin is one of the only AVPs with antiviral action against the Influenza A virus.

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. andreti* (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, Magainin-M2, Magainin-MW1, Magainin-P1, and Magainin-P2 (Ladrám 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; Ziari 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) (FLSGIVGMLKLF) 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 cell 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; Ladrám 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 (LPLJVGNLKLSS), 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 to develop resistance to these drugs (Piret and Bovin, 2011). Thus, Temporins can be considered preventive antitherpetic 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 (IPLRGAFINGRWDSQCHRFSNGAIACA) is a peptide secreted by the Indian frog belonging to the Ranidae family, *Hypophryllax 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 *Pelodyridaeae, Phyllomedusidae*, and *Pipidae* were grouped in Clade II. There is a close taxonomic relationship among the families *Phyllomedusidae* and *Pelodyridaeae*, 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 *Pelodyridaeae 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.](image-url)
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 highlights 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 desifin-5 (HDS) 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’ (SLDQNVTTFLDLLEYEMKLEAIKLEESYDIELKEL) 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’ (FLRLIKSKRFLVSFAK), which was designed as a peptide analog of the parent peptide Mucroporin (FLGFLPSLLGVL-VSAF) in the venom of the Lychea 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 Ebsavir. 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
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-1 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. Improved affinity with a strong and stable interaction of peptide molecules against SARS-CoV-2 Mpro may inhibit the entry into host cells and viruses due to coronavirus inability to contact ACE-2 receptors in the infection signaling process viral (Fakh 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 (Fakh, 2020) and can be used as an inhibitor of the virus spike glycoprotein (Satpathy, 2020).

Fakh (2020) performed an experiment through simulations of peptide-protein and protein-peptide 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 (ALWMTLKLKKVLKAAAKAALNAVLVGANA) and Dermaseptin-S9 (GLRSKILWLLLIMWQESNKFKKGNMLAALGAAADTISQGTQ), Dermaseptin-S4 (ALWMTLKLKKVLKAAAKAALNAVLVGANA), and Dermaseptin-S9 (GLRSKILWLLLIMWQESNKFKKKNMLAALGAAADTISQGTQ).

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 (ALWKTMLKKLGMTMALHAKGAALGAAADTISQGTQ), Dermaseptin-S4 (ALWMTLKLKKVLKAAAKAALNAVLVGANA), and Dermaseptin-S9 (GLRSKILWLLLIMWQESNKFKKKNMLAALGAAADTISQGTQ).

Dermaseptin-S9 peptide and the A chain of the spike protein of the SARS-CoV-2 showed a high 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 SARS-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 (GLFSVLGAVAKHLPHVPVIAEK) and 1.10 (GLISSVLGSKAVVHLPHPVPVIAEK) 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/segpred_mlr.pl) (Guernier 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 included 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 Brevicins, Maculatins, Esculentins, Temporins, and Uurumins 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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