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REVIEW

# Antibacterial properties of snake venom components

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**Abstract**
An increasing problem in the field of health protection is the emergence of drug-resistant and multi-drug-resistant bacterial strains. They cause a number of infections, including hospital infections, which currently available antibiotics are unable to fight. Therefore, many studies are devoted to the search for new therapeutic agents with bactericidal and bacteriostatic properties. One of the latest concepts is to search for this type of substances among toxins produced by venomous animals. In this approach, however, special attention is paid to snake venom because it contains molecules with antibacterial properties. Thorough investigations have shown that the phospholipases $A_2$ ($PLA_2$) and L-amino acids oxidases (LAAO), as well as fragments of these enzymes, are mainly responsible for the bactericidal properties of snake venoms. Some preliminary research studies also suggest that fragments of three-finger toxins (3FTx) are bactericidal. It has also been proven that some snakes produce antibacterial peptides (AMP) homologous to human defensins and cathelicidins. The presence of these proteins and peptides means that snake venoms continue to be an interesting material for researchers and can be perceived as a promising source of antibacterial agents.

**Keywords** Snake venom · Phospholipases $A_2$ · L-Amino acid oxidases · Antimicrobial properties

## Introduction

The most important clinical problem in the field of microbiology today is growing resistance to antibiotics in bacteria. According to the WHO, bacterial infections involving multi-drug-resistant (MDR) strains are one of the ten leading causes of death worldwide (Lopez et al. 2006). Moreover, antimicrobial resistance is considered to be one of the greatest threats to human health globally (Walker et al. 2009). Unfortunately, new examples of bacteria with antibiotic resistance appear every year. It is estimated that more than 90% of *Staphylococcus aureus* strains are resistant to β-lactam antibiotics (Panlilio 1992). Such resistance is shown, for example, by already long-known strains like methicillin-resistant *S. aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP) (Al Ahmadi et al. 2010). Recent studies have also shown that excessive use of antibiotics, such as vancomycin, may lead to developing vancomycin-intermediate (VISA)/vancomycin-resistant (VRSA) strains, like, for example, in the case of enterococci (Appelbaum 2006; Cázares-Domínguez et al. 2015). Other bacteria such as *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Acinetobacter*, *Salmonella* or *Enterococcus* have also developed several ways to resist antibiotics (Al Ahmadi et al. 2010). It is estimated that 23,000 and 25,000 people die every year in the USA and Europe, respectively, from infections caused by multidrug-resistant bacteria (CDC 2013; Blair et al. 2015). Presently existing and still appearing multiple-resistant strains increase the risk of bacterial infections, which become more and more threatening, as currently, we lack proper tools and drugs to combat them. Recently, many antimicrobials are at various stages of development and phases of clinical trials. However, it is still very clear that the discovery of new, potent antibacterial agents capable of overcoming drug resistance as well as the development of antibacterials with a new mechanism of action remains of the highest priority (Guardabassi and Kruse 2003; Ang et al. 2004; Roos 2004; de Lima et al. 2005; Al Ahmadi et al. 2010; Perumal Samy et al. 2017).

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## The pharmacological potential of snake venom

The composition of snake venom depends mainly on the species, but also on age, sex or type of food consumed (Koh et al. 2006). However, it should be mentioned that some systematic groups do not contain venomous snakes, e.g., boa or pythons. In some other groups, however, all the species classified there are venomous. Venomous snakes belong to following families: Viperidae (viperids, including vipers and rattlesnakes), Elapidae (elapids, including cobras, mambas and taipans), Hydrophiidae (sea snakes) and Colubridae (colubrids, although only some of them are venomous) (Gold et al. 2002; Warrell 2010; Burbrink and Crother 2011; Warrell 2019).

Snake venoms are complex mixtures of several families of protein-origin components that can be divided into 4 groups. The dominant are three-finger toxins (3FTx), phospholipases $A_2$ ($PLA_2$), snake venom metalloproteases (SVMP) and snake venom serine proteases (SVSP). The second group includes proteins commonly present in the venom, but in much smaller amounts: Kunitz peptides (KUN), Cysteine-Rich Secretory Proteins (CRiSP), L-amino acid oxidases (LAAO), C-type lectins (CTL), disintegrins (DIS), natriuretic peptides (NP). The third group contains proteins that are less commonly observed in venoms such as venom nerve growth factor (VNGF), vascular endothelial growth factor (VEGF), acetylcholinesterases, hyaluronidases, 5′-nucleotidases, phosphodiesterases (PDE), snake venom metalloprotease inhibitors and others. The last group contains rare proteins, among others: cobra venom factors (CVF), galactose-binding proteins, aminopeptidases or waprins. Of course, not all protein groups are found in all venomous snakes. For example, for elapids in general the most abundant proteins are phospholipases $A_2$ and 3FTx, however, this is not true for example for mambas. Their venom consists mostly of Kunitz peptides. On the other hand for viperids in general the most abundant groups are $PLA_2$s and proteases with different proportions of serine proteases and metalloproteases in different systematic groups (Tasoulis and Isbister 2017).

The toxins present in the venom exert a variety of biological effects such as neurotoxicity, myotoxicity, cardiotoxicity, hemorrhage, pro- and anti-coagulation, etc. (Perumal Samy et al. 2014a; Munawar et al. 2018). However, snake venoms as a complex mixture of proteins and peptides can also exhibit a wide range of pharmacological activities and may be used to develop new drugs with high therapeutic value (Koh et al. 2006; Waheed et al. 2017). The classic example in this field are the bradykinin-potentiating peptides (BPP), found in *Bothrops jararaca* venom, the inhibitors of the somatic angiotensin-converting enzyme (ACE) (Ferreira et al. 1970). On the basis of one of them, teprotide, the first active site-directed inhibitor of ACE was developed, which is currently used to treat human hypertension, namely captopril (Cushman and Ondetti 1991; Plosker and McTavish 1995). Past discoveries and developmental works proved that venom proteins can lead to production of drugs that are in clinical use and commercially generate billions of dollars. Besides the most famous example, captopril, there are others. For example eptifibatide is an antiplatelet drug with a cyclic heptapeptide structure based on the three amino acid sequence (Lys-Gly-Asp) found in barbourin, which is a disintegrin from *Sistrurus miliarius barbouri* venom (Fig. 1). Similar pharmacological profile can be seen in tirofiban, peptidomimetic agent based on the RGD sequence (Arg-Gly-Asp) from echistatin, protein from *Echis carinatus* venom (Diz-Küçükkaya and López 2018). There are also some examples of anticoagulant drugs available on the market namely: Reptilase (Batroxobin from *Bothrops atrox*), Defibrase (Moojenin from *Bothrops moojeni*) and Vivostat (serine protease from *B. moojeni*) (Waheed et al. 2017). Moreover, several venom-based compounds show promising pharmacological potential and currently undergo comprehensive investigation during clinical or preclinical studies. These are: cenderitide (Ichiki et al. 2019), Vipegitide (Lazarovici et al. 2019), antifibatide (Masias and Cataland2017), vicrostatin (Swenson et al. 2018), Dis*Ba*-01 (Danilucci et al. 2019), HCA—hemocoagulase agkistrodon (Li et al. 2018a), RPI-NM, and RPI-78M (Waheed et al. 2017). Also, the group of following compounds were currently withdrawn from the market: alfimeprase—potent fibrinolytic recombinant analog of metalloproteinase from *Agkistrodon contortrix contortrix* venom previously used for thrombolysis (Jones et al. 2001) and ancrod-serine protease from *Calloselasma rhodostoma* previously used as an anticoagulant agent (Nolan et al. 1976; Waheed et al. 2017).

## Snake venoms in drug design and development

Snake venoms are known to be a complicated mixture of proteins and peptides with great potential for drug design and development, which can ultimately lead to their clinical use. Unfortunately, the therapeutic use of peptide-origin drugs is problematic, especially due to their low bioavailability through the oral route, poor permeability, metabolic inactivation, the danger of proteolysis or enzymatic degradation, binding to plasma protein and finally, toxicity (Craik et al. 2013). Presently these limitations are being overcome through various approaches, for example, using antimicrobial peptides (AMP) externally in contact lenses coating

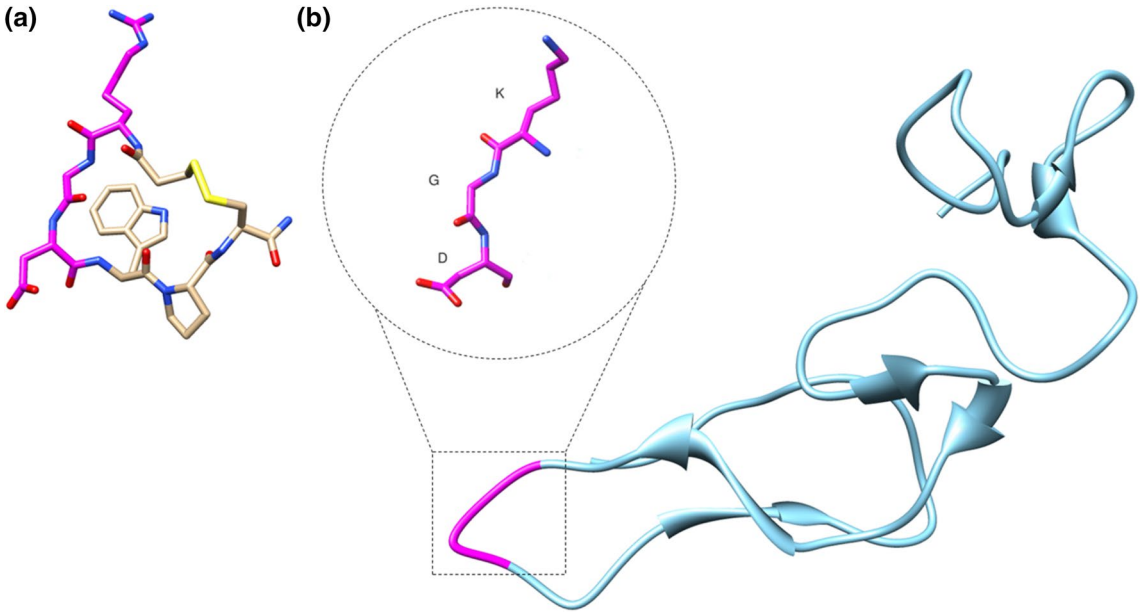


**Fig. 1** 3D structures of eptifibatide (PDB: 2VDN; Chain: C) and barbourin (PDB: 1Q7J; Model: 1). **a** Colored fragment of eptifibatide (in magenta) derive from (Lys-Gly-Asp) motif of barbourin and was introduced to the cyclic template to form functional heptapeptide. The side chain of lysine in eptifibatide was derivatized to improve the efficacy of the drug. **b** KGD sequence of barbourin (shown in magenta) was used as a template for several anti-platelet drug candidates (Lazarovici et al. 2019), (Chimera software)

(Dutta et al. 2014), using biocompatible carriers which enhance bioavailability (Lax and Meenan 2012; Maia et al. 2014) or encapsulation in biodegradable polymers (Anthony and Freda 2009). Snake venom toxins, from small peptides to large proteins, are very interesting, pharmacologically active compounds with wide chemical and functional variability, stability and specificity. They may inspire innovative discoveries including development of research tools or invention of new drugs like antibacterial and antitumor compounds. Currently, it is believed that pharmaceutical and biomedical research should lead to routine use of venom toxins as structural templates for the design and synthesis of novel and efficient therapeutic agents (de Oliveira-Junior et al. 2013; Almeida et al. 2017, 2018).

Currently, hundreds of therapeutic peptides are under development and at different stages of clinical tests (Kaspar and Reichert 2013; Uhlig et al. 2014). Majority of them are involved in cancer: e.g., disintegrins with antiangiogenesis effect or LAAOs inducing apoptosis (Li et al. 2018b) and cardiovascular diseases treatment, besides the examples mentioned in the previous paragraph, also, e.g., natriuretic peptides and ion channel blockers (Koh and Kini 2012). However, there are also peptides tested for pain treatment (e.g., mambalgin from *Dendroaspis polylepis* venom) (Diochot et al. 2012) and infectious diseases, for example LAAOs from *Trimeresurus stejnegeri* venom inhibit infection and replication of HIV-1 virus (Zhang et al. 2003) and LAAOs from *Bothrops jararaca* have antiviral (Dengue virus) and antiprotozoal (trypanocidal and leishmanicide) activities (Sant'Ana et al. 2008).

The use of venom components for drug discovery is rapidly increasing, though it is still mostly an unrealized prospect due to recurrent technical bottlenecks that represent venom exploration (Lewis and Garcia 2003). It is estimated that although all animal venoms consist of over 40 million proteins and peptides, only a very small fraction of them are known (Escoubas and King 2009). The advent and development of -omic techniques has led to discovery of an increasing number of toxins with known sequences and structures, which are available for biomedical and biotechnological exploitation (King 2011). The future direction of venom research with the use of modern 'omics' techniques such as genomics, transcriptomics and proteomics should lead to identification and characterization of new therapeutic molecules from animal venoms. According to the authorities in this field, the key for the search of novel antimicrobial molecules is the characterization of previously unexplored and rare animal venoms, as they may be the new source of antibacterial molecules (Perumal Samy et al. 2017).

## The antibiotic potential of snake venom

Antimicrobial agents are used in medicine to treat infections caused by microbes from different classes of pathogenic organisms, namely viruses, protozoa, fungi and

bacteria, including among others, rickettsia, mycoplasma and chlamydia. Among them, bacteria are the largest and most diverse group of pathogenic microorganisms (Rouault 2004). Antimicrobial agents normally used to treat bacterial infections are divided into two groups: bacteriostatics and bactericidals. Bacteriostatic agents arrest the growth of bacteria (e.g., sulphonamides, tetracycline, chloramphenicol), bactericidal agents, on the other hand, kill bacterial cells through disruption of cell wall/membrane function (Chao et al. 2013). The effectiveness of both, existing drugs and venom components, depend on the type of bacteria. For example, the bactericidal activity of *Bothrops alternatus* venom is higher against *Escherichia coli* and *S. aureus* versus *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Bustillo et al. 2008).

Recent studies prove that many venoms and venom components produced by different venomous animals show potential antibacterial activity. These include snake (Perumal Samy et al. 2007; Al Ahmadi et al. 2010; Ferreira et al. 2011; Perumal Samy et al. 2014b), spider (Haeberli et al. 2000; Budnik et al. 2004; Kozlov et al. 2006; Benli and Yigit 2008), scorpion (Conde et al. 2000; Torres-Larios et al. 2002), honeybee (EL-Feel et al. 2015; Leandro et al. 2015) and wasp venoms (Jalaei et al. 2014).

The antibiotic potency of snake venom is well known and documented and it is mainly dependent on the venom composition as well as on the specific bacterial types (de Oliveira Junior et al. 2013). The major clinical challenge in developing novel antibiotics is the design of new, less toxic molecules that effectively combat the recent emergence of MDR clinical pathogens such as *S. aureus*, *E. coli* and enterococci. Therefore, the majority of research is devoted to attempts to break through resistance of these bacteria. Examples of venom tested for antibacterial properties are summarized in Table 1. Both Viperidae and Elapidae venoms have been tested on numerous occasions and much of the obtained data give very promising results indicating antimicrobial activity in vitro that can rival currently used antibiotics. It has also been noted that viperid venoms exhibit a broader spectrum of antibacterial activity against different bacterial strains (Perumal Samy et al. 2007). However, based on the evidence, elapids venoms and their components may also represent valuable resource for future development of novel human therapeutics useful in fighting with bacterial infections (Birrell et al. 2007).

## Snake venom components with antimicrobial properties

Generally, components of snake venoms can be divided into peptide-origin and non-peptide-origin. The first group is discussed in the second paragraph of the article and it may constitute more than 90% of venom's dry weight, while the second group consists of low molecular weight organic compounds such as carbohydrates, serotonin, histamine, citrate, and nucleosides; and inorganic ions such as calcium, cobalt, magnesium, copper, iron, and potassium. The toxic effect of venom, both in the context of victim bite and potential antibacterial effect, is caused by the components of the first group (Izidoro et al. 2014).

### Phospholipases $A_2$

One of the most common groups of enzymes present in both elapid and viperid venoms are phospholipases $A_2$, which can be divided into basic and acidic $PLA_2$s. Basic $PLA_2$s are usually responsible for major toxic effects induced by snake venoms, while acidic $PLA_2$s tend to have a lower toxicity (Doley et al. 2010). The sv$PLA_2$s (snake venom $PLA_2$s) are very interesting enzymes due to their potential for being therapeutic lead molecules with antimicrobial properties against enveloped bacteria, viruses, fungi, parasites, and protozoa (Perumal Samy et al. 2007, 2012). Basic $PLA_2$ from *Crotalus durissus terrificus* has strong bactericidal effects against both Gram-positive and -negative bacteria (Toyama et al. 2003). An acidic $PLA_2$ from *Porthidium nasutum* venom has bactericidal activity against *S. aureus* with MIC (minimal inhibitory concentration) value of 32 µg/ml (Vargas et al. 2012). A myotoxic $PLA_2$ (MjTX-II) from *B. moojeni* demonstrates antimicrobial activity against *E. coli* (Okubo et al. 2012). Phospholipase $A_2$ from *Crotalus adamanteus*, called toxin-II (CaTx-II) has antibacterial properties against *S. aureus* and *Burkholderia pseudomallei* and also inhibits *Enterobacter aerogenes* growth causing disintegration of cell wall, by the generation of pores in membrane. Moreover, it has been shown that this protein can promote wound healing (Perumal Samy et al. 2014b). Membrane permeabilization is also caused by basic myotoxin crotamine from *C. durissus terrificus* and, what is interesting, this effect is limited to prokaryotic cells because it acts without any haemolytic effects (Oguiura et al. 2011). New $PLA_2$ from *Walterinnesia aegyptia* venom has antimicrobial properties against several human pathogenic strains (Ben Bacha et al. 2018) and $PLA_2$ from *Pseudonaja textilis* is able to inhibit the growth of *S. aureus* (Perumal Samy et al. 2007) and *Burkholderia pseudomallei* (Perumal Samy et al. 2006). The mechanism of action in this case is associated with pore formation and membrane damaging effects on the bacterial cell wall without any cytotoxic effects on lung and skin fibroblast cells (Perumal Samy et al. 2014b).

What is also interesting, peptides formed from a sv$PLA_2$ fragmentation are also able to interact with lipopolysaccharide (LPS), particularly the lipid A component of *S. aureus,* causing membrane permeabilization, and exerting bactericidal effects (Perumal Samy et al. 2014b). Cysteine-rich

**Table 1** Summary of previously tested venoms for antibacterial properties

| Snake species | Bacterial species | References |
|---|---|---|
| *Montivipera bornmuelleri* | *Staphylococcus aureus*<br>*Morganella morganii* | Accary et al. (2014) |
| *Echis carinatus* | *Staphylococcus aureus* | Al Ahmadi et al. (2010) |
| *Walterinnesia aegyptia*<br>*Echis pyramidum*<br>*Echis coloratus*<br>*Cerastes gasperettii*<br>*Naja arabica* | *Staphylococcus aureus* | Al-Asmari et al. (2015) |
| *Walterinnesia aegyptia*<br>*Echis pyramidum*<br>*Echis coloratus*<br>*Naja arabica* | *Enterococcus faecalis*<br>*Escherichia coli*<br>*Pseudomonas aeruginosa* | Al-Asmari et al. (2015) |
| *Bothrops alternatus* | *Escherichia coli*<br>*Staphylococcus aureus* | Bustillo et al. (2008) |
| *Bothrops jararaca* | *Staphylococcus aureus*<br>*Enterococcus faecalis* | Ferreira et al. (2011) |
| *Calloselasma rhodostoma*<br>*Bothrops atrox* | *Staphylococcus epidermidis*<br>*Staphylococcus aureus*<br>*Enterococcus faecalis* | Ferreira et al. (2011) |
| *Vipera ammodytes* | *Proteus vulgaris*<br>*Staphylococcus aureus*<br>*Staphylococcus epidermidis*<br>*Enterococcus faecium* | Iğci et al. (2016) |
| *Montivipera bornmuelleri* | *Salmonella enteritidis*<br>*Staphylococcus aureus* | Jaoudeh et al. (2017) |
| *Montivipera latifii* | *Bacillus subtilis*<br>*Staphylococcus aureus* | Moridikia et al. (2018) |
| *Crotalus adamanteus*<br>*Daboia russelii russelii*<br>*Pseudechis australis*<br>*Pseudechis guttata*<br>*Agkistrodon halys*<br>*Bitis rhinoceros* | *Burkholderia pseudomallei* | Perumal Samy et al. (2006) |
| *Daboia russelii russelii*<br>*Echis carinatus*<br>*Bitis rhinoceros*<br>*Bitis arietans*<br>*Pseudechis australis*<br>*Naja naja naja* | *Staphylococcus aureus* | Perumal Samy et al. (2007) |
| *Ophiophagus hannah* | *Staphylococcus aureus* | Rangsipanuratn et al. (2019) |
| *Naja naja* | *Escherichia coli*<br>*Pseudomonas aeruginosa*<br>*Vibrio cholerae*<br>*Staphylococcus aureus*<br>*Bacillus subtilis* | Sachidananda et al. (2007) |
| *Calloselasma rhodostoma*<br>*Ophiophagus hannah* | *Staphylococcus aureus* | San et al. (2010) |
| *Bitis arietans*<br>*Pseudechis australis*<br>*Cerastes cerastes*<br>*Naja nigricollis*<br>*Naja naja naja*<br>*Vipera lebetina*<br>*Echis carinatus* | *Staphylococcus aureus* | Shebl et al. (2012) |
| *Naja nigricollis*<br>*Naja naja naja* | *Pseudomonas aeruginosa* | Shebl et al. (2012) |

**Table 1** (continued)

| Snake species | Bacterial species | References |
|---|---|---|
| *Pseudechis australis*<br>*Cerastes cerastes*<br>*Naja nigricollis*<br>*Naja naja naja*<br>*Vipera lebetina*<br>*Echis carinatus* | *Escherichia coli* | Shebl et al. (2012) |
| *Pseudechis australis*<br>*Cerastes cerastes*<br>*Naja naja naja* | *Salmonella typhimurium* | Shebl et al. (2012) |
| *Montivipera xanthina* | *Staphylococcus aureus* | Yalcın et al. (2013) |

AMPs in particular may have a broad spectrum of antimicrobial properties. They are characterized by flexibility of structure and positive charge, which are essential for the enrichment of antibacterial activity caused by hydrophobic attraction to bacterial membrane with negatively charged components (Perumal Samy et al. 2017). Different cationic peptides derived from svPLA$_2$s from *Bothrops asper* present antimicrobial activity against *Klebsiella pneumoniae*, fight peritonitis induced by *Salmonella enterica* in mice and cause membrane permeabilization of *S. aureus* (Santamaria et al. 2005). But what is most important, these peptides, which are composed of 10- to 22-odd amino acids, derived from the carboxy terminus of the svPLA$_2$s, are less toxic for eukaryotic cells and more bactericidal than the parent molecules. That is why this type of natural peptides and others may become a base for novel drugs design and lead to the production of new drugs with potential therapeutic value in the near future (White 2000; Koh et al. 2006).

Among PLA$_2$ family, there is a subgroup which can induce tissue damage by mechanisms independent of catalysis. These proteins have a single mutation, changing aspartate residue at position 49 for a lysine residue, and are called Lys49 PLA$_2$s. This substitution prevents the coordination of $Ca^{2+}$ ions in the binding loop, leading to loss of enzymatic activity (Delatorre et al. 2011). Although they do not hydrolyze membrane phospholipids, they have antimicrobial activity against a variety of pathogenic microorganisms (Páramo et al. 1998; Stábeli et al. 2006). It is believed that not a catalytic reaction but the distinctive primary structure consisting of a combination of hydrophobic and cationic residues in the C-terminal region of the molecule is responsible for antimicrobial activity by the destabilization and perturbation of biological membranes (Díaz et al. 1991, 2001). Several synthetic peptides were designed, based on such C-terminal sequence of 13 amino acids from *B. asper* myotoxin II, which is a homolog of Lys49 PLA$_2$ (Fig. 2). In fact, one of

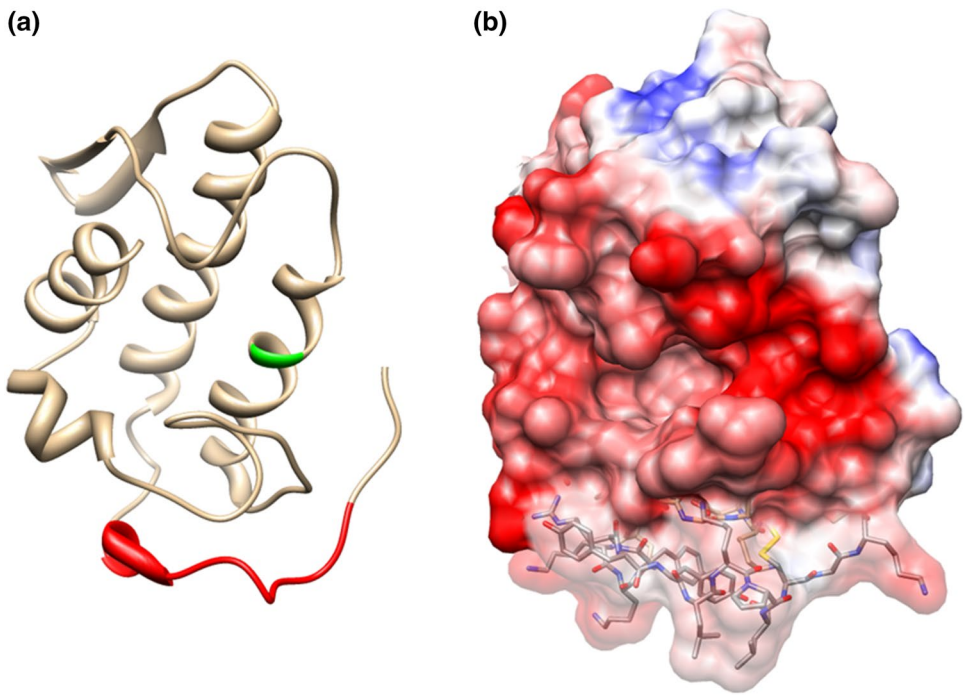


**Fig. 2** Different representations of the monomer of myotoxin II from *B. asper* (PDB: 1CLP; Chain: A). **a** Ribbon visualization of secondary structures of MTX-II (Lys49—in green; C-terminal sequence with bactericidal activity—in red). **b** Surface visualization of MTX-II with Coulombic Surface Coloring (areas with high electron density, which therefore are more negative are blue; white surfaces is neutral, while red color indicates regions with a positive charge). The figure shows that 13 amino acids AMP from myotoxin II has visible cationic nature, which is perceived as important for its overall bactericidal activity (Chimera software)

the derivatives exhibit very high bactericidal, fungicidal and even antitumor activity with low toxicity towards eukaryotic cells (Won and Ianoul 2009). Small peptides designed on the base of primary structure of Lys49 phospholipase $A_2$, namely CoaTx-II, from *Crotalus oreganus abyssus* have antibacterial effect against drug-resistant clinical isolates. It was proven that presence of charged and aromatic amino acids plays an important role in interaction of peptides with bacterial cell membrane (Almeida et al. 2018). The antibacterial evaluation of LmutTX, Lys49 $PLA_2$ from *Lachesis muta muta* snake venom and synthetic peptides designed on its base show promising activity against Gram-negative and Gram-positive bacteria (Diniz-Sousa et al. 2018). Also other C-terminal, cationic peptides derived from Lys49 $PLA_2$s have been evaluated for their microbicidal and anti-tumor potential (Páramo et al. 1998; Murillo et al. 2007; Costa et al. 2008), presenting promising results. Furthermore, the synthetic peptides show high specificity, potent action at low dose, low immunogenicity, high diffusion to tissues, and relatively easy chemical synthesis with the possibility of modifications, such as the use of D-amino acids or cyclization. All these advantages make synthetic peptides, developed on the base of snake venom proteins, a very promising alternative to traditional drugs (Almeida et al. 2018).

## L-Amino acid oxidases

The second major group of venom enzymes responsible for antimicrobial properties is L-amino acid oxidases (LAAO). They are usually homodimeric proteins with covalently linked cofactors (FAD or FMN), however, their structures, molecular masses, and isoelectric points can be significantly different. Concentration of snake venom LAAOs varies between systematic groups and affects venom toxicity and its color. Those enzymes catalyze the oxidative deamination of hydrophobic and aromatic amino acids in a wide range of pHs and temperatures. In the first step of the reaction the amino acid substrate is oxidized to an imino acid, with a simultaneous reduction of the cofactor. In the second step the imino acid undergoes nonenzymatic hydrolysis, yielding $\alpha$-keto acid and ammonia. In order for the next reaction to occur, it is necessary to close the catalytic cycle by regenerating the cofactor. Reoxidizing of cofactor takes place in the presence of molecular oxygen and thus generates hydrogen peroxide. It is believed that the production of hydrogen peroxide opens perspectives for new applications of these enzymes as bactericidal, antiviral, and antitumor agents, making them a promising biotechnological agent. In prey's body they induce changes in platelet function, which cause local effects on plasma clotting disorders among other things. But in vitro, LAAOs also trigger apoptosis in various cell lines and show antimicrobial and antiparasitic activity (Izidoro et al. 2014).

Bactericidal effect of snake venom L-amino acid oxidases was reported in the case of several species of both viperids (e.g., Ciscotto et al. 2009; Costa Torres et al. 2010; Vargas et al. 2013) and elapids (e.g., Samel et al. 2008; Lee et al. 2011). Generally, snake venom LAAOs exhibit various levels of antibacterial activity against different bacteria strains. L-Amino acid oxidase from *P. australis* venom is 17.5 times more effective than tetracycline against *Aeromonas hydrophila* on a molar basis (Stiles et al. 1999). The venom of *Bothrops leucurus* inhibits *S. aureus* growth in a dose-dependent manner, with a MIC of 25 µg/ml. LAAOs from *C. adamanteus* and *B. asper* exert antibacterial activity against *S. aureus* and *Proteus mirabilis* same as svLAAO from *Bothrops* venoms (Tempone et al. 2001; Izidoro et al. 2006; Costa Torres et al. 2010). Another LAAO from *Bothrops pirajai* controls the growth of *Pseudomonas aeruginosa* and *Escherichia coli* (Izidoro et al. 2006). And as in the case of $PLA_2$s, also small fragments of LAAO show enhanced antimicrobial activity. These small peptides could be promising candidates in the new antibiotics design (Okubo et al. 2012).

There are at least two hypotheses about antibacterial activity of LAAOs. The first is related to the oxidized form of the cofactor of the enzyme (FAD or FMN). This cofactor interacts with L-amino acids which can then act on nucleic acids, proteins, and the plasma membrane (Izidoro et al. 2014). The second involves hydrogen peroxide which, after interaction with the bacterial membrane, can provoke lipoperoxidation (Toyama et al. 2006), DNA fragmentation (Braga et al. 2008), and in consequence cell death. It is also probable that LAAO can directly oxidize amino acids in proteins (Ande et al. 2008). Generally it is believed that the most probable mechanism of bactericidal activity of LAAOs involves oxidative stress in the bacteria cell, triggering disorganization and permeabilization of the plasma membrane and finally death of the cell, all caused by presence of hydrogen peroxide in the reaction medium (Izidoro et al. 2014).

## Antimicrobial peptides

*Staphylococcus aureus* and the coagulase-negative *S. epidermidis,* colonizing the nose and skin, are the most common commensal bacteria causing infections in humans and other mammals. The infection develops only when the protective layer of the human epithelium is breached and mechanisms of host immunity fail. These mechanisms include antimicrobial peptides (AMP) present on the skin and in the sweat. They are the first line of innate immune defenses on the human skin and also form part of the mechanisms by which bacteria are eliminated in the neutrophil phagosome after phagocytosis. AMPs in humans belong to two major groups: defensins and cathelicidins and many of them are active against staphylococci (Joo and Otto 2015).

Similarly to humans, other animals including snakes, produce small cationic antimicrobial peptides (cAMP) called cathelicidins. These peptides have a broad-spectrum of antimicrobial activity against a wide variety of bacteria, enveloped viruses, and fungi (Perumal Samy et al. 2017). Transcriptomic analyses of venom glands of several species (*Naja atra*, *Bungarus fasciatus* and *Ophiophagus hannah*) reveal that snakes' cathelicidins are highly homologous with AMPs found in lysosomes of cells in the immune system and have strong antibacterial properties (Wang et al. 2008, 2011; Zhao et al. 2008). BF-30, cathelicidin-type peptide derived from *B. fasciatus* is very effective against diverse antibiotic-resistant bacteria, including those that cause wounds (MRSA) (Chen et al. 2011a). It was shown that it reduces number of bacteria at the wound, but also prevents inflammation and accelerates wound healing (Zhou et al. 2011; Du et al. 2015). Cathelicidin (OH-CATH) from *Ophiophagus hannah* and its analogs exert strong antibacterial and weak hemolytic activity. They are very effective against *Acinetobacter* spp., including multi-drug-resistant *Acinetobacter baumannii* (MRAB) and methicillin-resistant *Staphylococcus aureus* (MRSA) and their effectiveness is higher than that of the 9 routinely used antibiotics (Zhao et al. 2018). The myotoxin from *Crotalus durissus* venom called crotamine is on the other hand structurally related to beta-defensin antimicrobial peptides (AMP) found in vertebrate animals (Oguiura et al. 2011). Whole crotamine-myotoxin family shows high degree of homology (60–80%) with beta-defensin (Mancin et al. 1998; Nicastro et al. 2003) which makes them a very interesting subject for future research.

## Other proteins

$PLA_2$s, LAAOs and AMP are the most widely described groups of proteins with antibacterial properties. But there are also single descriptions of peptides and proteins from other groups, which are very promising and open the way to completely new discoveries. Most of antibacterial peptides act by binding, interacting and finally disrupting the lipid plasma membrane. For example, toxin gamma from cobra *Naja nigricollis,* belonging to three-finger toxin (3FTx) family, increases membrane permeability of *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria which leads to the bactericidal effects of this protein. The direct molecular activity of this protein is binding to the major membrane constituents for Gram-negative and positive bacteria, lipopolysaccharide (LPS) and lipoteichoic acid (LTA), respectively. Destabilization of LPS layer and inhibition of LTA biosynthesis causes bactericidal effects (Chen et al. 2011c). Another 3FTx example—cardiotoxin 3 from *Naja naja atra*—has the same mechanism of action, but its activity is greater against *S. aureus* than against *E. coli* (Chen et al. 2011b). Different venom peptides and proteins interact with bacteria membrane components by electrostatic and ionic interaction. It was observed that most of these toxins have positive molecular net charge and are able to bind with anionic and zwitterionic phospholipid particles. This mechanism of action is distinctive for other 3FTxs, namely cardiotoxins from *N. naja atra* venom (Kao et al. 2009). On the basis of *N. naja atra* cardiotoxin 1 (CTX-1), also belonging to the three-finger toxins family, a series of small peptides was designed. The developed peptides consist of sequences that normally build the tip and subsequent β-strand of the first "finger" of this toxin. Thanks to that, new peptides had microbicidal activity towards strains of Gram-positive and Gram-negative bacteria of original protein with the lack of general toxicity (Fig. 3) (Sala et al. 2018).

Another example of an interesting protein is omwaprin originating from *Oxyuranus microlepidotus* venom. It is an acidic protein belonging to the waprin family (whey acidic proteins) and it has selective antibacterial properties against Gram-positive bacteria. Its action is based on damaging of the cell membrane of bacteria, which in consequence leads to the leakage of cell contents and cell death. Interestingly,

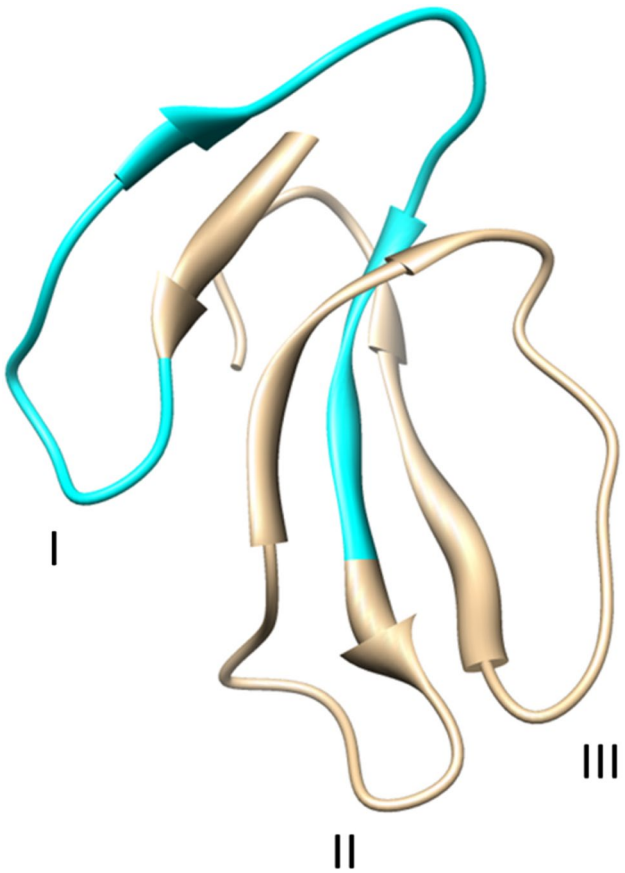


**Fig. 3** The structure of cardiotoxin 1 from *N. atra* (PDB: 2CDX; Model: 1). Individual fingers of 3FTx are labeled in the figure. The sequence (KLIPIASKTCPAGKNLCYKM) that was used to design several AMPs is shown in cyan. (Chimera software)

this protein does not damage human cells, which has been proven in tests on erythrocytes (Nair et al. 2007).

Similar to 3FTxs and waprins, C-type lectin-like proteins are an example of venom components without enzymatic activity. Representatives of this group often show contradictory actions: some induce platelet aggregation and agglutination, while others inhibit this effect. Both purified lectins, such as the one from *Bothrops leucurus* venom, namely BlL, and lectin homologs, have antimicrobial properties. Mentioned BlL protein from *B. leucurus* is effective against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis* (Nunes Edos et al. 2011) and the protein from *Bothrops jararacussu* acts against *Staphylococcus aureus* (Klein et al. 2015) whereas homologs of convulxin from *Crotalus durissus terrificus* decrease the growth of *Xanthomonas axonopodis* and *Clavibacter michiganensis michiganensis* (Rádis-Baptista et al. 2006).

A final example of a venom component with antibacterial properties is enzyme AHM from *Agkistrodon halys* belonging to metalloproteinase family. This protein is very effective against *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus and* multi-drug resistant *Burkholderia pseudomallei*. The mechanism of action is associated with damage to the membranes, wrinkling of cell surfaces, leakage of cell contents and formation of vesicles on cell surfaces, with the consequence that the membrane and wall lose their integrity (Perumal Samy et al. 2008).

## Conclusions

In the era of great threat posed by antibiotic-resistant strains of bacteria, we face a great challenge which is to develop modern methods of antibacterial therapies. One of the promising trends is the search for compounds with antibacterial potential among venom components. It has been repeatedly proven that both whole snake venom and its individual components, or even their fragments, have the desired properties and are therefore a potential source of new antibiotics. This approach is all the more promising as there are known examples of the development of effective drugs based on proteins and peptides derived from snake venom.

Moreover, much attention should be devoted to understanding the different mechanisms responsible for the antibacterial activity of venom-based drugs. This will certainly enable finding new promising drug templates as well as optimizing existing structures. Therefore, the identification of new venom-origin agents, combined with alternative routes of administration, developed in recent years, make them a very promising line of research with great potential for the future. With increased approval of peptide-based drugs and advances in peptide-associated technologies, they are becoming more and more medically significant.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.



## References

Accary C, Hraoui-Bloquet S, Hamze M, Mallem Y, El Omar F, Sabatier J-M, Desfontis J-C, Fajloun Z (2014) Protein content analysis and antimicrobial activity of the crude venom of *Montivipera bornmuelleri*; a viper from Lebanon. Infect Disord Drug Targets 14(1):49–55

Al Ahmadi AJ, Fathi B, Jamshidi A, Zolfagharian H, ZareMirakabbadi A (2010) Investigation of the antibacterial effect of venom of the Iranian snake *Echis carinatus*. Iran J Vet Sci Technol 2:93–100. https://doi.org/10.22067/veterinary.v2i2.8369

Al-Asmari AK, Abbasmanthiri R, Abdo Osman NM, Siddiqui Y, Al-Bannah FA, Al-Rawi AM, Al-Asmari SA (2015) Assessment of the antimicrobial activity of few Saudi Arabian snake venoms. Open Microbiol J 9:18–25. https://doi.org/10.2174/1874285801509010018

Almeida JR, Resende LM, Watanabe RK, Corassola VC, Huancahuire-Vega S, Caldeira CAdS, Coutinho-Neto A, Soares AM, Vale N, Gomes PADC, Marangoni S, Calderon LdA, Silva SLD (2017) Snake venom peptides and low mass proteins: molecular tools and therapeutic agents. Curr Med Chem 24:3254–3282. https://doi.org/10.2174/0929867323666161028155611

Almeida JR, Mendes B, Lancellotti M, Marangoni S, Vale N, Passos Ó, Ramos MJ, Fernandes PA, Gomes P, Da Silva SL (2018) A novel synthetic peptide inspired on Lys49 phospholipase A2 from *Crotalus oreganus abyssus* snake venom active against multidrug-resistant clinical isolates. Eur J Med Chem 149:248–256. https://doi.org/10.1016/j.ejmech.2018.02.05

Ande SR, Fussi H, Knauer H, Murkovic M, Ghisla S, Fröhlich KU, Macheroux P (2008) Induction of apoptosis in yeast by L-amino acid oxidase from the Malayan pit viper *Calloselasma rhodostoma*. Yeast 25(5):349–357. https://doi.org/10.1002/yea.1592

Ang JY, Ezike E, Asmar BI (2004) Antibacterial resistance. Indian J Pediatr 71(3):229–239

Anthony L, Freda PU (2009) From somatostatin to octreotide LAR: evolution of a somatostatin analogue. Curr Med Res Opin 25:2989–2999. https://doi.org/10.1185/03007990903328959

Appelbaum PC (2006) The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12:16–23. https://doi.org/10.1111/j.1469-0691.2006.01344.x

Ben Bacha A, Alonazi MA, Elshikh MS, Karray A (2018) A novel bactericidal homodimeric $PLA_2$ group-I from *Walterinnesia aegyptia* venom. Int J Biol Macromol 117:1140–1146. https://doi.org/10.1016/j.ijbiomac.2018.06.024

Benli M, Yigit N (2008) Antibacterial activity of venom from funnel web spider *Agelena labyrinthica* (Araneae: Agelenidae). J Venom Anim Toxins Incl Trop Dis 17(4):641–650. https://doi.org/10.1590/S1678-91992008000400007

Birrell GW, Earl STH, Wallis TP, Masci PP, de Jersey J, Gorman JJ, Lavin MF (2007) The diversity of bioactive proteins in

Australian snake venoms. Mol Cell Proteom 6:973–986. https://doi.org/10.1074/mcp.M600419-MCP200

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13(1):42–51. https://doi.org/10.1038/nrmicro3380

Braga MDM, Martins AMC, Amora DN, de Menezes DB, Toyama MH, Toyama DO, Marangoni S, Alves CD, Barbosa PS, de Sousa Alves R, Fonteles MC, Monteiro HS (2008) Purification and biological effects of L-amino acid oxidase isolated from *Bothrops insularis* venom. Toxicon 51(2):199–207. https://doi.org/10.1016/j.toxicon.2007.09.003

Budnik BA, Olsen JV, Egorov TV, Anisimova VE, Galkina TG, Musolyamov AK, Grishin EV, Zubarev RA (2004) De novo sequencing of antimicrobial peptides isolated from the venom glands of the wolf spider *Lycosas ingoriensis*. J Mass Spectrom 39:193–201. https://doi.org/10.1002/jms.577

Burbrink FT, Crother BI (2011) Evolution and taxonomy of snakes. In: Aldridge RD, Sever DM (eds) Reproductive biology and phylogeny of snakes, 1st edn. CRC Press, Boca Raton, pp 19–53

Bustillo S, Leiva LC, Merino L, Acosta O, de Kier Joffé EB, Gorodner JO (2008) Antimicrobial activity of *Bothrops alternatus* venom from the Northeast of Argentine. Rev Latinoam Microbiol 50(3):79–82

Cázares-Domínguez V, Cruz-Córdova A, Ochoa SA, Escalona G, Arellano-Galindo J, Rodríguez-Leviz A, Hernández-Castro R, López-Villegas EO, Xicohtencatl-Cortes J (2015) Vancomycin tolerant, methicillin-resistant *Staphylococcus aureus* reveals the effects of vancomycin on cell wall thickening. PLoS ONE 10:e0118791. https://doi.org/10.1371/journal.pone.0118791

CDC Centers for Disease Control and Prevention (2013) Antibiotic resistance threats in the United States. http://www.cdc.gov/drugresistance/threat-report-2013/. Accessed 08 July 2019

Chao MC, Kieser KJ, Minami S, Mavrici D, Aldridge BB, Fortune SM, Alber T, Rubin EJ (2013) Protein complexes and proteolytic activation of the cell wall hydrolase RipA regulate septal resolution in mycobacteria. PLoS Pathog 9(2):e1003197. https://doi.org/10.1371/journal.ppat.1003197

Chen W, Yang B, Zhou H, Sun L, Dou J, Qian H, Huang W, Mei Y, Han J (2011a) Structure–activity relationships of a snake cathelicidin-related peptide, BF-15. Peptides 32:2497–2503. https://doi.org/10.1016/j.peptides.2011.10.005

Chen LW, Kao PH, Fu YS, Lin SR, Chang LS (2011b) Membrane-damaging activity of Taiwan cobra cardiotoxin 3 is responsible for its bactericidal activity. Toxicon 58(1):46–53. https://doi.org/10.1016/j.toxicon.2011.04.021

Chen LW, Kao PH, Fu YS, Hu WP, Chang LS (2011c) Bactericidal effect of *Naja nigricollis* toxin γ is related to its membrane-damaging activity. Peptides 32(8):1755–1763. https://doi.org/10.1016/j.peptides.2011.06.026

Ciscotto P, Machado de Avila RA, Coelho EA, Oliveira J, Diniz CG, Farías LM, de Carvalho MA, Maria WS, Sanchez EF, Borges A, Chávez-Olórtegui C (2009) Antigenic, microbicidal and antiparasitic properties of an L-amino acid oxidase isolated from *Bothrops jararaca* snake venom. Toxicon 53(3):330–341. https://doi.org/10.1016/j.toxicon.2008.12.004

Conde R, Zamudio FZ, Rodtiguez MH, Possani LD (2000) Scorpine, an anti-malaria and anti-bacterial agent purified from scorpion venom. FEBS Lett 471:165–168. https://doi.org/10.1016/s0014-5793(00)01384-3

Costa Torres AF, Dantas RT, Toyama MH, DizFilho E, Zara FJ, Rodrigues de Queiroz MG, Pinto Nogueira NA, Rosa de Oliveira M, de Oliveira Toyama D, Monteiro HS, Martins AM (2010) Antibacterial and antiparasitic effects of *Bothrops marajoensis* venom and its fractions: phospholipase $A_2$ and L-amino acid oxidase. Toxicon 55(4):795–804. https://doi.org/10.1016/j.toxicon.2009.11.013

Costa TR, Menaldo DL, Oliveira CZ, Santos-Filho NA, Teixeira SS, Nomizo A, Fuly AL, Monteiro MC, de Souza BM, Palma MS, Stabeli RG, Sampaio SV, Soares AM (2008) Myotoxic phospholipases $A_2$ isolated from *Bothrops brazili* snake venom and synthetic peptides derived from their C-terminal region: cytotoxic effect on microorganism and tumor cells. Peptides 29:1645–1656. https://doi.org/10.1016/j.peptides.2008.05.021

Craik DJ, Fairlie DP, Liras S, Price D (2013) The future of peptide-based drugs. Chem Biol Drug Des 81:136–147. https://doi.org/10.1111/cbdd.12055

Cushman DW, Ondetti MA (1991) History of the design of captopril and related inhibitors of angiotensin converting enzyme. Hypertension 17:589–592

Danilucci TM, Santos PK, Pachane BC, Pisani GFD, Lino RLB, Casali BC, Altei WF, Selistre-de-Araujo HS (2019) Recombinant RGD-disintegrin Dis*Ba*-01 blocks integrin $\alpha_v\beta_3$ and impairs VEGF signaling in endothelial cells. Cell Commun Signal 17(1):27. https://doi.org/10.1186/s12964-019-0339-1

de Lima DC, Alvarez Abreu P, de Freitas CC, Santos DO, Borges RO, Dos Santos TC, Mendes Cabral L, Rodrigues CR, Castro HC (2005) Snake venom: any clue for antibiotics and CAM? Evid Based Complement Alternat Med 2:39–47. https://doi.org/10.1093/ecam/neh063

de Oliveira Junior NG, e Silva Cardoso MH, Franco OL (2013) Snake venoms: attractive antimicrobial proteinaceous compounds for therapeutic purpose. Cell Mol Life Sci 70(24):4645–4658. https://doi.org/10.1007/s00018-013-1345-x

Delatorre P, Rocha BAM, Santi-Gadelha T, Gadelha CAA, Toyama MH, Cavada BS (2011) Crystal structure of Bn IV in complex with myristic acid: a Lys49 myotoxic phospholipase $A_2$ from *Bothrops neuwiedi* venom. Biochimie 93:513–518. https://doi.org/10.1016/j.biochi.2010.11.003

Díaz C, Gutiérrez J, Lomonte B, Gené J (1991) The effect of myotoxins isolated from *Bothrops* snake venoms on multilamellar liposomes: relationship to phospholipase A 2, anticoagulant and myotoxic activities. Biochim Biophys Acta 1070:455–460. https://doi.org/10.1016/0005-2736(91)90086-n

Díaz C, León G, Rucavado A, Rojas N, Schroit J, Gutiérrez JM (2001) Modulation of the susceptibility of human erythrocytes to snake venom myotoxic phospholipases $A_2$: role of negatively charged phospholipids as potential membrane binding sites. Arch Biochem Biophys 391:56–64. https://doi.org/10.1006/abbi.2001.2386

Diniz-Sousa R, Caldeira CAS, Kayano AM, Paloschi MV, Pimenta DC, Simões-Silva R, Ferreira AS, Zanchi FB, Matos NB, Grabner FP, Calderon LA, Zuliani JP, Soares AM (2018) Identification of the molecular determinants of the antibacterial activity of LmutTX, a Lys49 phospholipase $A_2$ homologue isolated from *Lachesis muta muta* Snake venom (Linnaeus, 1766). Basic Clin Pharmacol Toxicol 22(4):413–423. https://doi.org/10.1111/bcpt.12921

Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay AS, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. Nature 490:552–555. https://doi.org/10.1038/nature11494

Diz-Küçükkaya R, López JA (2018) Acquired disorders of platelet function. In: Hoffman R, Benz EJ, Silberstein LE, Heslop HE, Weitz JI, Anastasi J, Salama ME, Abutalib SA (eds) Hematology, 7th edn. Elsevier, Amsterdam, pp 1932–1943

Doley R, Zhou X, Kini RM (2010) Snake venom phospholipase A 2 enzymes. In: Mackessy SP (ed) Handbook of venoms and toxins of reptiles. CRC Press, Taylor & Francis Group, Boca Raton, pp 173–206

Du H, Samuel RL, Massiah MA, Gillmor SD (2015) The structure and behavior of the NA-CATH antimicrobial peptide with

liposomes. Biochim Biophys Acta 1848:2394–2405. https://doi.org/10.1016/j.bbamem.2015.07.006
Dutta D, Ozkan J, Willcox MDP (2014) Biocompatibility of antimicrobial melamine lenses. Optom Vis Sci 91:570–581. https://doi.org/10.1097/OPX.0000000000000232
El-Feel MA, Abdel-Rahman EH, Abed Al-Fattah MA (2015) Antibacterial activity of bee venom collected from *Apis mellifera* carniolan pure and hybrid races by two collection methods. Int J Curr Microbiol App Sci 4:141–149
Escoubas P, King GF (2009) Venomics as a drug discovery platform. Expert Rev Proteom 6:221–224. https://doi.org/10.1586/epr.09.45
Ferreira SH, Bartelt DC, Greene LJ (1970) Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. Biochemistry 9(13):2583–2593. https://doi.org/10.1021/bi00815a005
Ferreira BL, Santos OD, dos Santos AL, Rodrigues CR, de Freitas CC, Cabral LM, Castro HC (2011) Comparative analysis of Viperidae venoms antibacterial profile: a short communication for proteomics. Evid Based Complement Alternat Med 2011:960267. https://doi.org/10.1093/ecam/nen052
Gold BS, Dart RC, Barish RA (2002) Bites of venomous snakes. N Engl J Med 347(5):347–356. https://doi.org/10.1056/NEJMra013477
Guardabassi L, Kruse H (2003) Overlooked aspects concerning development and spread of antimicrobial resistance. Expert Rev Anti Infect Ther 1(3):359–362 **PMID: 15482133**
Haeberli S, Kuhn-Nentwing L, Schaller J, Nentwig W (2000) Characterization of antibacterial activity of peptides isolated from the venom of the spider *Cupiennius salei*. Toxicon 38:373–380. https://doi.org/10.1016/S0041-0101(99)00167-1
Ichiki T, Dzhoyashvili N, Burnett JC Jr (2019) Natriuretic peptide based therapeutics for heart failure: Cenderitide: a novel first-in-class designer natriuretic peptide. Int J Cardoil 281:166–171. https://doi.org/10.1016/j.ijcard.2018.06.002
Iğci N, Nalbantsoy A, Erkan LG, Akça GY, Yalçın HT, Yalçın M, Göçmen B (2016) Screening of cytotoxic, anti-angiogenic, anti-tumorogenic and antimicrobial activities of Anatolian Vipera ammodytes (Nose-horned viper) venom. Turkish J Biochem 41:483–491
Izidoro LFM, Ribeiro MC, Souza GRL, Sant'Anna CD, Hamaguchi A, Homsi-Brandeburgo MI, Goulart LR, Beleboni RO, Nomizo A, Sampaio SV, Soares AM, Rodrigues VM (2006) Biochemical and functional characterization of an L-amino acid oxidase isolated from *Bothrops pirajai* snake venom. Bioorg Med Chem 14(20):7034–7043. https://doi.org/10.1016/j.bmc.2006.06.025
Izidoro LF, Sobrinho JC, Mendes MM, Costa TR, Grabner AN, Rodrigues VM, da Silva SL, Zanchi FB, Zuliani JP, Fernandes CF, Calderon LA, Stábeli RG, Soares AM (2014) Snake venom L-amino acid oxidases: trends in pharmacology and biochemistry. Biomed Res Int 2014:196754. https://doi.org/10.1155/2014/196754
Jalaei J, Fazeli M, Rajaian H, Shekarforoush SS (2014) In vitro antibacterial effect of wasp (*Vespa orientalis*) venom. J Venom Anim Toxins Incl Trop Dis 20(20):22. https://doi.org/10.1186/1678-9199-20-22
Jaoudeh CA, Hraoui-Bloquet S, Sadek R, Rizk R, Hleihel W (2017) Antibacterial effect of the *Montivipera bornmuelleri* crude venom against *Salmonella enteritidis* and *Staphylococcus aureus*. RJPBCS 8(2):137
Jones G, Ronk M, Mori F, Zhang Z (2001) Disulfide structure of alfimeprase: a recombinant analog of fibrolase. Protein Sci 10(6):1264–1267. https://doi.org/10.1110/ps.110101
Joo H-S, Otto M (2015) Mechanisms of resistance to antimicrobial peptides in *Staphylococci*. Biochim Biophys Acta 1848:3055–3061. https://doi.org/10.1016/j.bbamem.2015.02.009
Kao PH, Lin SR, Chang LS (2009) Differential binding to phospholipid bilayers modulates membrane-damaging activity of *Naja naja atra* cardiotoxins. Toxicon 54(3):321–328. https://doi.org/10.1016/j.toxicon.2009.04.024
Kaspar AA, Reichert JM (2013) Future directions for peptide therapeutics. Drug Discov Today 18:807–817. https://doi.org/10.1016/j.drudis.2013.05.011
King GF (2011) Venoms as a platform for human drugs: translating toxins into therapeutics. Expert Opin Biol Ther 11:1469–1484. https://doi.org/10.1517/14712598.2011.621940
Klein RC, Fabres-Klein MH, de Oliveira LL, Feio RN, Malouin F, Ribon Ade O (2015) A C-type lectin from *Bothrops jararacussu* venom disrupts staphylococcal biofilms. PLoS ONE 10(3):e0120514. https://doi.org/10.1371/journal.pone.0120514
Koh CY, Kini RM (2012) From snake venom toxins to therapeutics–cardiovascular examples. Toxicon 59(4):497–506. https://doi.org/10.1016/j.toxicon.2011.03.017
Koh DC, Armugam A, Jeyaseelan K (2006) Snake venom components and their applications in biomedicine. Cell Mol Life Sci 63:3030–3041. https://doi.org/10.1007/s00018-006-6315-0
Kozlov SA, Vassilevski AV, Feofanov AY, Surovoy DV, Karpunin EV, Grishin E (2006) Latarcins antimicrobial and cytolytic peptides from venom of the spider *Lachesana tarabaevi* (Zodariidae) that exemplify biomolecular diversity. J Biol Chem 281(30):20983–20992. https://doi.org/10.1074/jbc.M602168200
Lax R, Meenan C (2012) Challenges for therapeutic peptides part 1: on the inside, looking out. Innov Pharm Technol 42:54–56
Lazarovici P, Marcinkiewicz C, Lelkes PI (2019) From snake venom's disintegrins and C-type lectins to anti-platelet drugs. Toxins 11(5):303. https://doi.org/10.3390/toxins11050303
Leandro LF, Mendes CA, Casemiro LA, Vinholis AH, Cunha WR, de Almeida R, Martins CH (2015) Antimicrobial activity of apitoxin, melittin and phospholipases A 2 of honey bee (*Apis mellifera*) venom against oral pathogens. An Acad Bras Cienc 87:147–155. https://doi.org/10.1590/0001-3765201520130511
Lee ML, Tan NH, Fung SY, Sekaran SD (2011) Antibacterial action of a heat-stable form of L-amino acid oxidase isolated from king cobra (*Ophiophagus hannah*) venom. Comp Biochem Physiol C 153(2):237–242. https://doi.org/10.1016/j.cbpc.2010.11.001
Lewis RJ, Garcia ML (2003) Therapeutic potential of venom peptides. Nat Rev Drug Discov 2:790–802. https://doi.org/10.1038/nrd1197
Li H, Huang Y, Wu X, Wu T, Cao Y, Wang Q, Qiu Y, Fu W, Zhang Q, Pang J (2018a) Effects of hemocoagulase agkistrodon on the coagulation factors and its procoagulant activities. Drug Des Dev Ther 12:1385–1398. https://doi.org/10.2147/DDDT.S159210
Li L, Huang J, Lin Y (2018b) Snake venoms in cancer therapy: past, present and future. Toxins 10(9):346. https://doi.org/10.3390/toxins10090346
Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJL (2006) Global burden of disease and risk factors. IBRD/TheWorld Bank and Oxford University Press, Washington, DC
Maia FR, Barbosa M, Gomes DB, Vale N, Gomes P, Granja PL, Barrias CC (2014) Hydrogel depots for local co-delivery of osteoinductive peptides and mesenchymal stem cells. J Control Release 189:1873–4995. https://doi.org/10.1016/j.jconrel.2014.06.030
Mancin AC, Soares AM, Andriao-Escarso SH, Faca VM, Green LJ, Zuccolotto S, Pela IR, Giglio JR (1998) The analgesic activity of crotamine, a neurotoxin *from Crotalus durissus terrificus* (South American rattlesnake) venom: a biochemical and pharmacological study. Toxicon 36:1927–1937. https://doi.org/10.1016/S0041-0101(98)00117-2
Masias C, Cataland SR (2017) Novel therapies in thrombotic thrombocytopenic purpura. Res Pract Thromb Haemost 2(1):19–26. https://doi.org/10.1002/rth2.12066
Moridikia A, Zargan J, Sobati H, Goodarzi HR, Hajinourmohamadi A (2018) Anticancer and antibacterial effects of Iranian

viper (*Vipera latifii*) venom; an in vitro study. J Cell Physiol 233(9):6790–6797. https://doi.org/10.1002/jcp.26428

Munawar A, Ali SA, Akrem A, Betzel C (2018) Snake venom peptides: tools of biodiscovery. Toxins 10(11):E474. https://doi.org/10.3390/toxins10110474

Murillo LA, Lan C-Y, Agabian NM, Larios S, Lomonte B (2007) Fungicidal activity of a phospholipase-A 2-derived synthetic peptide variant against *Candida albicans*. Rev Esp Quimioter 20:330–333 **PMID: 18080030**

Nair DG, Fry BG, Alewood P, Kumar PP, Kini RM (2007) Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom proteins. Biochem J 402:93–104. https://doi.org/10.1042/BJ20060318

Nicastro G, Franzoni L, de Chiara C, Mancin AC, Giglio JR, Spisni A (2003) Solution structure of crotamine, a Na-channel affecting toxin from *Crotalus durissus terrificus* venom. Eur J Biochem 270:1969–1979. https://doi.org/10.1046/j.1432-1033.2003.03563.x

Nolan C, Hall LS, Barlow GH (1976) Ancrod, the coagulating enzyme from Malayan Pit Viper (*Agkistrodon rhodostoma*) venom. Methods Enzymol 45:205–213. https://doi.org/10.1016/s0076-6879(76)45020-6

Nunes Edos S, de Souza MA, Vaz AF, Santana GM, Gomes FS, Coelho LC, Paiva PM, da Silva RM, Silva-Lucca RA, Oliva ML, Guarnieri MC, Correia MT (2011) Purification of a lectin with antibacterial activity from *Bothrops leucurus* snake venom. Comp Biochem Physiol B Biochem Mol Biol 159(1):57–63. https://doi.org/10.1016/j.cbpb.2011.02.001

Oguiura N, Boni-Mitake M, Affonso R, Zhang G (2011) In vitro antibacterial and hemolytic activities of crotamine, a small basic myotoxin from rattlesnake *Crotalus durissus terrificus*. J Antibiot 64:327–331. https://doi.org/10.1038/ja.2011.10

Okubo BM, Silva ON, Migliolo L, Gomes DG, Porto WF, Batista CL, Ramos CS, Holanda HH, Dias SC, Franco OL, Moreno SE (2012) Evaluation of an antimicrobial L-amino acid oxidase and peptide derivates from *Bothropoides mattogrosensis* pitviper venom. PLoS ONE 7(3):e33639. https://doi.org/10.1371/journal.pone.0033639

Panlilio AL (1992) Methicillin-resistant *S. aureus* in U.S. hospitals. Infect Control Hosp Epidemiol 3:582–586. https://doi.org/10.1086/646432

Páramo L, Lomonte B, Pizarro-cerdá J, Bengoechea JA, Gorvel JP, Moreno E (1998) Bactericidal activity of Lys49 and Asp49 myotoxic phospholipases A 2 from *Bothrops asper* snake venom: synthetic Lys49 myotoxin II-(115-129)-peptide identifies its bactericidal region. Eur J Biochem 253:452–461. https://doi.org/10.1046/j.1432-1327.1998.2530452.x

Perumal Samy R, Pachiappan A, Gopalakrishnakone P, Thwin MM, Hian YE, Chow TKV, Bow H, Weng JT (2006) In vitro antibacterial activity of natural toxins and animal venoms tested against *Burkholderia pseudomallei*. BMC Infect Dis 6(100):1–16. https://doi.org/10.1186/1471-2334-6-100

Perumal Samy R, Gopalakrishnakone P, Thwin MM, Chow TK, Bow H, Yap EH, Thong TW (2007) Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase $A_2$ enzymes. J Appl Microbiol 102:650–659. https://doi.org/10.1111/j.1365-2672.2006.03161.x

Perumal Samy R, Gopalakrishnakone P, Chow VT, Ho B (2008) Viper metalloproteinase (Agkistrodon halys pallas) with antimicrobial activity against multi-drug resistant human pathogens. J Cell Physiol 216(1):54–68. https://doi.org/10.1002/jcp.21373

Perumal Samy R, Gopalakrishnakone P, Stiles BG, Girish KS, Swamy SN, Hemshekhar M, Tan KS, Rowan EG, Sethi G, Chow VTK (2012) Snake venom phospholipases $A_2$: a novel tool against bacterial diseases. Curr Med Chem 19:6150–6162. https://doi.org/10.2174/092986731120906615O

Perumal Samy R, Manikandan J, Sethi G, Franco OL, Okonkwo JC, Stiles BG, Chow VTC, Gopalakrishnakone P, Qahtani MA (2014a) Snake venom proteins: development into antimicrobial and wound healing agents. Mini-Rev Org Chem 11:1–14. https://doi.org/10.2174/1570193X1101140402100131

Perumal Samy R, Kandasamy M, Gopalakrishnakone P, Stiles BG, Rowan EG, Becker D, Shanmugam MK, Sethi G, Chow VT (2014b) Wound healing activity and mechanisms of action of an antibacterial protein from the venom of the eastern diamondback rattlesnake (*Crotalus adamanteus*). PLoS ONE 9:e80199. https://doi.org/10.1371/journal.pone.0080199

Perumal Samy R, Stiles BG, Franco OL, Sethi G, Lim LHK (2017) Animal venoms as antimicrobial agents. Biochem Pharmacol 134:127–138. https://doi.org/10.1016/j.bcp.2017.03.005

Plosker GL, McTavish D (1995) Captopril. A review of its pharmacology and therapeutic efficacy after myocardial infarction and in ischaemic heart disease. Drugs Aging 7:226–253. https://doi.org/10.2165/00002512-199507030-00007

Rádis-Baptista G, Moreno FB, de Lima Nogueira L, Martins AM, Toyama DO, Toyama MH, Cavada BS, Azevedo WF, Yamane T (2006) Crotacetin, a novel snake venom C-type lectin homolog of convulxin, exhibits an unpredictable antimicrobial activity. Cell Biochem Biophys 44:412–423. https://doi.org/10.1385/CBB:44:3:412

Rangsipanuratn W, Sandee A, Daduang J, Janwithayanuchit I (2019) Antibacterial activity of snake venoms against bacterial clinical isolates. Pharm Sci Asia 46(2):80–87. https://doi.org/10.29090/psa.2019.01.018.0003

Roos KL (2004) Emerging antimicrobial-resistant infections. Arch Neurol 61:1512–1514. https://doi.org/10.1001/archneur.61.10.1512

Rouault TA (2004) Pathogenic bacteria prefer heme. Science 305:1577–1578. https://doi.org/10.1126/science.1102975

Sachidananda MK, Murari SK, Channe Gowda D (2007) Characterization of an antibacterial peptide from Indian cobra (*Naja naja*) venom. J Venom Anim Toxins Incl Trop Dis 13(2):446–461. https://doi.org/10.1590/S1678-91992007000200004

Sala A, Cabassi CS, Santospirito D, Polverini E, Flisi S, Cavirani S, Taddei S (2018) Novel *Naja atra* cardiotoxin1 (CTX-1) derived antimicrobial peptides with broad spectrum activity. PLoS ONE 13(1):e0190778. https://doi.org/10.1371/journal.pone.0190778

Samel M, Tõnismägi K, Rönnholm G, Vija H, Siigur J, Kalkkinen N, Siigur E (2008) L-Amino acid oxidase from *Naja naja oxiana* venom. Comp Biochem Physiol B 149(4):572–580. https://doi.org/10.1016/j.cbpb.2007.11.008

San TM, Vejayan J, Shanmugan K, Ibrahim H (2010) Screening of antimicrobial activity of venoms from snakes commonly found in Malaysia. J Appl Sci 10:2328–2332. https://doi.org/10.3923/jas.2010.2328.2332

Santamaría C, Larios S, Quirós S, Pizarro-Cerda J, Gorvel J, Lomonte B, Moreno E (2005) Bactericidal and antiendotoxic properties of short cationic peptides derived from a snake venom Lys49 phospholipase A2. Antimicrob Agents Chemother 49:1340–1345. https://doi.org/10.1128/AAC.49.4.1340-1345.2005

Sant'Ana CD, Menaldo DL, Costa TR, Godoy H, Muller VD, Aquino VH, Albuquerque S, Sampaio SV, Monteiro MC, Stábeli RG, Soares AM (2008) Antiviral and antiparasite properties of an L-amino acid oxidase from the snake *Bothrops jararaca*: cloning and identification of a complete cDNA sequence. Biochem Pharmacol 76(2):279–288. https://doi.org/10.1016/j.bcp.2008.05.003

Shebl RI, Mohamed AF, Ali AE, Amin MA (2012) Antimicrobial profile of selected snake venoms and their associated enzymatic activities. Br Microbiol Res J 2:251–263. https://doi.org/10.9734/BMRJ/2012/2091

Stábeli RG, Amui SF, Sant'Ana CD, Pires MG, Nomizo A, Monteiro MC, Romão PRT, Guerra-Sá R, Vieira CA, Giglio JR, Fontes

MRM, Soares AM (2006) *Bothrops moojeni* myotoxin-II, a Lys49-phospholipase A2 homologue: an example of function versatility of snake venom proteins. Comp Biochem Physiol C 142:371–381. https://doi.org/10.1016/j.cbpc.2005.11.020

Stiles BG, Sexton FW, Weinstein SA (1999) Antibacterial effects of different snake venoms: purification and characterization of antibacterial proteins from *Pseudechis australis* (Australian king brown or mulga snake) venom. Toxicon 29(9):1129–1141. https://doi.org/10.1016/0041-0101(91)90210-I

Swenson S, Minea RO, Tuan CD, Thein TZ, Chen TC, Markland FS (2018) A novel venom-derived peptide for brachytherapy of glioblastoma: preclinical studies in mice. Molecules 23(11):2918. https://doi.org/10.3390/molecules23112918

Tasoulis T, Isbister GK (2017) A review and database of snake venom proteomes. Toxins 9(9):290. https://doi.org/10.3390/toxins9090290

Tempone AG, Andrade HF Jr, Spencer PJ, Lourenço CO, Rogero JR, Nascimento N (2001) *Bothrops moojeni* venom kills *Leishmania* spp. with hydrogen generated by its L-amino acid oxidase. Biochem Biophys Res Commun 280(3):620–624. https://doi.org/10.1006/bbrc.2000.4175

Torres-Larios A, Gurrola GB, Zamudio FZ, Possani LD (2002) Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. Eur J Biochem 267:5023–5031. https://doi.org/10.1046/j.1432-1327.2000.01556.x

Toyama MH, de Oliveira DG, Beriam LOS, Novello JC, Rodrigues-Simioni L, Marangoni S (2003) Structural, enzymatic and biological properties of new $PLA_2$ isoform from *Crotalus durissus terrificus* venom. Toxicon 41(8):1033–1038. https://doi.org/10.1016/S0041-0101(03)00085-0

Toyama MH, Toyama DDO, Passero LFD, Laurenti MD, Corbett CE, Tomokane TY, Fonseca FV, Antunes E, Joazeiro PP, Beriam LO, Martins MA, Monteiro HS, Fonteles MC (2006) Isolation of a new L-amino acid oxidase from *Crotalus durissus cascavella* venom. Toxicon 47:47–57. https://doi.org/10.1016/j.toxicon.2005.09.008

Uhlig T, Kyprianou T, Martinelli FG, Oppici C, Heiligers D, Hills D, Calvo XR, Verhaert P (2014) The emergence of peptides in the pharmaceutical business: from exploration to exploitation. EuPA Open Proteomics 4:58–69. https://doi.org/10.1016/j.eupro t.2014.05.003

Vargas LJ, Londoño M, Quintana JC, Rua C, Segura C, Lomonte B, Núñez V (2012) An acidic phospholipase A 2 with antibacterial activity from *Porthidium nasutum* snake venom. Comp Biochem Physiol B 161(4):341–347. https://doi.org/10.1016/j.cbpb.2011.12.010

Vargas LJ, Quintana JC, Pereañez JA, Núñez V, Sanz L, Calvete J (2013) Cloning and characterization of an antibacterial L-amino acid oxidase from *Crotalus durissus cumanensis* venom. Toxicon 64:1–11. https://doi.org/10.1016/j.toxicon.2012.11.027

Waheed H, Moin SF, Choudhary MI (2017) Snake venom: from deadly toxins to life-saving therapeutics. Curr Med Chem 24(17):1874–1891. https://doi.org/10.2174/0929867324666170605091546

Walker B, Barrett S, Polasky S, Galaz V, Folke C, Engstrom G, Ackerman F, Arrow K, Carpenter S, Chopra K, Daily G, Ehrlich P, Hughes T, Kautsky N, Levin S, Maler K, Shogren J, Vincent J, Xepapadeas T, Zeeuw A (2009) Environment. Looming global-scale failures and missing institutions. Science 325:1345–1346. https://doi.org/10.1126/science.1175325

Wang Y, Hong J, Liu X, Yang H, Liu R, Wu J, Wang A, Lin D, Lai R (2008) Snake cathelicidin from *Bungarus fasciatus* is a potent peptide antibiotics. PLoS ONE 3:e3217. https://doi.org/10.1371/journal.pone.0003217

Wang Y, Zhang Z, Chen L, Guang H, Li Z, Yang H, Li J, You D, Yu H, Lai R (2011) Cathelicidin-BF, a snake cathelicidin-derived antimicrobial peptide, could be an excellent therapeutic agent for *Acne vulgaris*. PLoS ONE 6:e22120. https://doi.org/10.1371/journal.pone.0022120

Warrell DA (2010) Snake bite. Lancet 375:77–88. https://doi.org/10.1016/S0140-6736(09)61754-2

Warrell DA (2019) Venomous bites, stings, and poisoning: an update. Infect Dis Clin N Am 33:17–38. https://doi.org/10.1016/j.idc.2018.10.001

White J (2000) Bites and stings from venomous animals: a global overview. Therap Drug Monit 22(1):65–68 **PMID: 10688262**

Won A, Ianoul A (2009) Interactions of antimicrobial peptide from C-terminus of myotoxin II with phospholipid mono- and bilayers. Biochim Biophys Acta 1788(10):2277–2283. https://doi.org/10.1016/j.bbamem.2009.07.012

Yalcın HT, Ozen MO, Gocmen B, Nalbantsoy A (2013) Effect of Ottoman viper (*Montivipera xanthina* (Gray, 1849)) venom on various cancer cells and on microorganisms. Cytotechnology 66(1):87–94. https://doi.org/10.1007/s10616-013-9540-z

Zhang YJ, Wang JH, Lee WH, Wang Q, Liu H, Zheng YT, Zhang Y (2003) Molecular characterization of *Trimeresurus stejnegeri* venom L-amino acid oxidase with potential anti-HIV activity. Biochem Biophys Res Commun 309(3):598–604. https://doi.org/10.1016/j.bbrc.2003.08.044

Zhao H, Gan T, Liu X, Jin Y, Lee W, Shen J, Zhang Y (2008) Identification and characterization of novel reptile cathelicidins from elapid snakes. Peptides 29:1685–1691. https://doi.org/10.1016/j.peptides.2008.06.008

Zhao F, Lan XQ, Du Y, Chen PY, Zhao J, Zhao F, Lee WH, Zhang Y (2018) King cobra peptide OH-CATH30 as a potential candidate drug through clinic drug-resistant isolates. Zool Res 39(2):87–96. https://doi.org/10.24272/j.issn.2095-8137.2018.025

Zhou H, Dou J, Wang J, Chen L, Wang H, Zhou W, Li Y, Zhou C (2011) The antibacterial activity of BF-30 in vitro and in infected burned rats is through interference with cytoplasmic membrane integrity. Peptides 32:1131–1138. https://doi.org/10.1016/j.peptides.2011.04.002