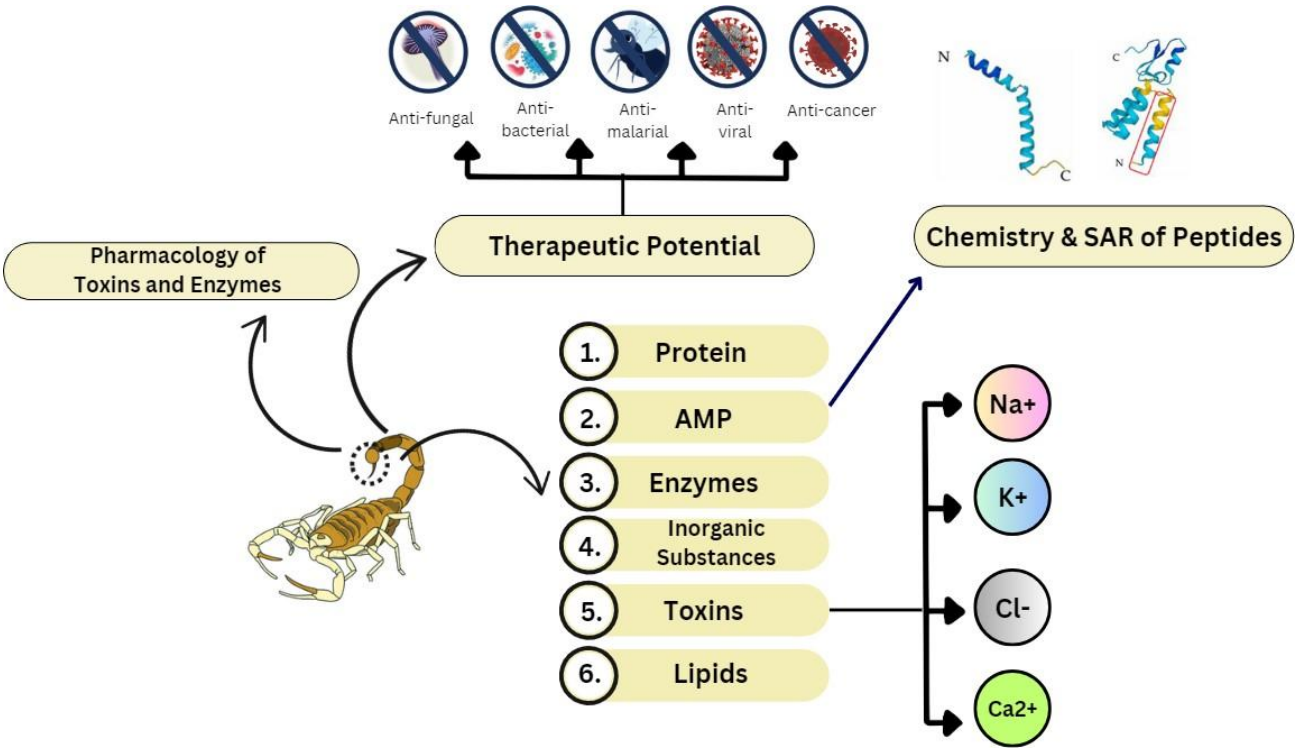
CHEMISTRY OF SCORPION VENOM AND ITS MEDICINAL POTENTIAL

Khetal Surana, Shashwat Singh, Aditya Lade, Nitin Arote, Shreerang Joshi*

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Mumbai, India - 400019.

Abstract:

Scorpion venom comprises a unique mix of peptides, proteins, enzymes, and other compounds, showing great medical potential. This review examines its chemical composition, categorising toxins into groups like α-toxins, β-toxins, potassium channel toxins (KTxs), calcins, and antimicrobial peptides, each with distinct pharmacological effects. It also highlights potential therapeutic applications in cancer treatment and antimicrobial therapies. While using scorpion venom for medical purposes is promising, challenges such as toxicity and non-selectivity toward healthy cells remain. Continued research is crucial to fully harness its therapeutic potential and address these issues for innovative treatment developments.



Keywords: Toxins, Enzymes, Antimicrobial Peptides, Structure-Activity Relationship, Pre-Clinical, Anti-Cancer, Anti-Microbial

Abbreviations: NaTxs, sodium channel toxins; KTxs, potassium channel toxins; AMP, anti- microbial peptides; aa, amino acid; NDBP, non-disulfide bridged peptides; Nbs, nanobodies; SCN, short chain neurotoxin

Introduction

Scorpions are exciting creatures that measure around 2.5 inches and are one of the most primitive, land-dwelling arthropods alive today. They are the members of the Arachnida class, and they have existed for around 430 million years, which shows their resilience and survival skills. They are highly venomous and thrive in various environments, including tropical and temperate forests, caves, savannas, and grasslands. [1] Having been around for an impressive 430 million years, they exemplify resilience and survival. As members of the Arachnida class, they are known for being highly venomous. So far, over 1,500 different types of scorpions have been recognised across the globe. [2] They are mysterious animals with a vast and complex genome for coding proteins with around 32.016 genes. The venom is divided into three superfamilies with calcins, cysteine-stabilized α/β motifs, and non-disulfide bridged peptides; around 800 active polypeptides have been categorised in these families. In addition to these peptides, scorpion venom also hosts amines, enzymes (larger proteins), free amino acids, nucleotides, various inorganic salts, and lipids, showcasing its intricate composition.

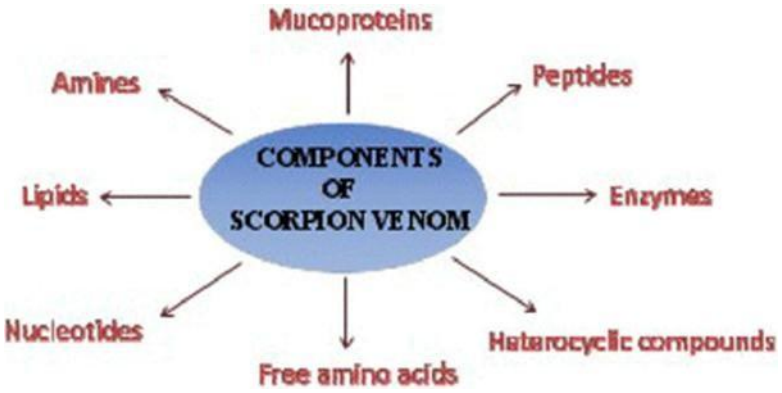


Fig. 1 Components of Scorpion Venom [3]

Venom derived from scorpion is gaining attention worldwide for its therapeutic applications, and it's a mix of peptides, enzymes, proteins, and nucleotides.

The toxins also influence the ion channels in the human body, which affects how ions such as sodium, potassium, calcium, and chloride move in and out of cells. Short-chain peptides mainly obstruct potassium and chloride channels, whereas long-chain peptides are aimed at sodium and calcium channels. [4] Studies have shown that toxins affecting sodium channels are especially effective in mammals, including humans. The peptides derived from venom have different structural features, with around 26% featuring disulfide bridges, which enhances its stability and ability to work in various therapeutic contexts. Because of this, they are being researched as possible treatments for numerous conditions. For example, they have potential applications in managing cardiovascular diseases, HIV, various microbial conditions, and epilepsy and tackling autoimmune disorders and multiple cancers, such as lung, oral, kidney, breast, and prostate. Additionally, they have been investigated for their effectiveness against brain tumours, neuroblastoma, and pancreatitis.

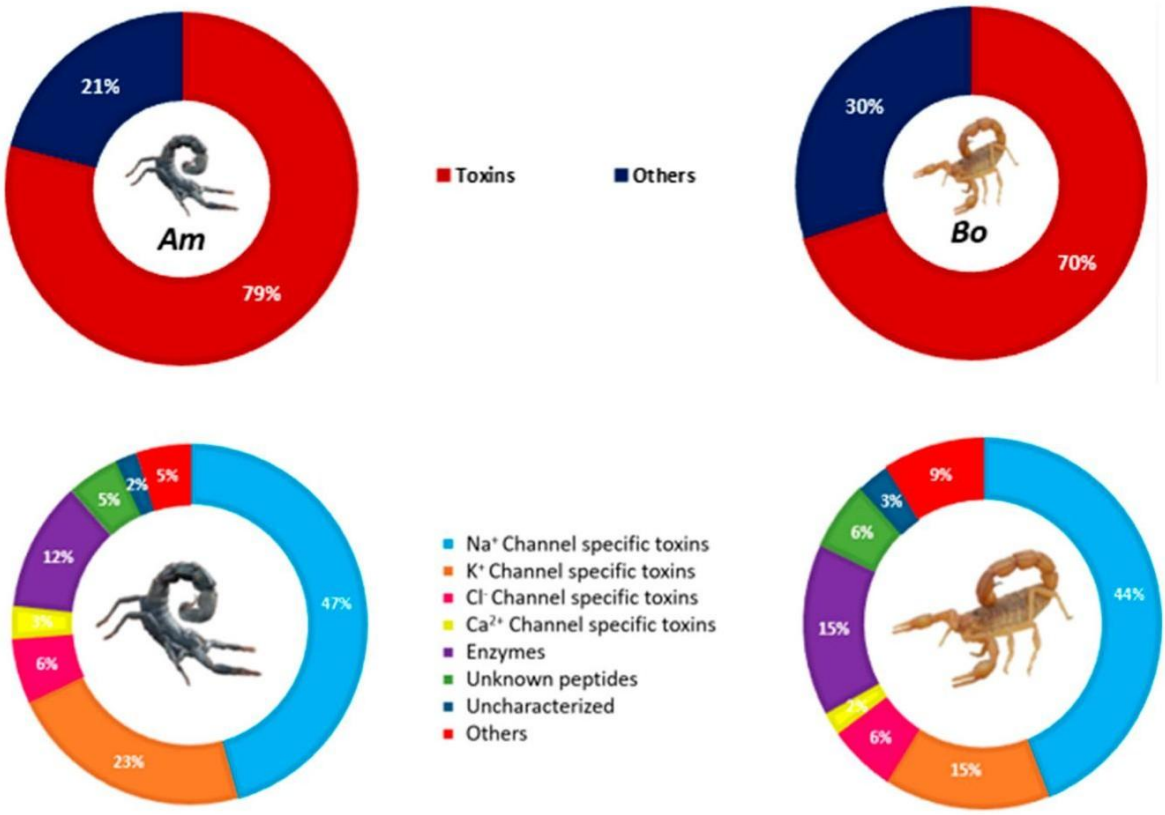


Fig. 2 *Androctonus mauritanicus* and *Buthus occitanus* venom components relative abundance [5]

This review paper will discuss scorpion venom's chemical composition and its toxins' chemistry and pharmacology. We also look after the venom's therapeutic application, the structure-activity relationship of peptides, and the extensive anti-cancer research using scorpion-derived venom, with several bioactive compounds currently in pre-clinical testing.

Composition of Scorpion Venom:

Scorpion venom serves two primary purposes: it helps them catch their food and protects them from threats. [6] This venom is a complicated blend of various compounds, such as neurotoxic peptides, histamine, mucopolysaccharides, serotonin, phospholipase, hyaluronidase, and enzyme inhibitors. Researchers have utilised advanced techniques like fractionation, chromatography, and peptide sequencing to study the components found in the venoms of scorpions, snakes, and spiders. [7] The unique characteristics of these venoms can be determined by pinpointing particular peptide toxins and analysing their molecular structures. Interestingly, they serve as highly selective antagonists for voltage-gated channels, allowing ions like potassium, sodium, and calcium to pass through. [8] The neurotoxins and proteins interfere with the body's normal functions, affecting the central and peripheral nervous systems. This disruption can lead to changes in muscle activity as well. [7] Scorpion toxins could be categorised based on their structure, how they act, and where they bind to different channels or their subtypes. Every category encompasses a range of peptides sourced from the venom of different scorpion species. [9] Remarkably, these toxins' pharmacological effects align closely with their peptide families' structural characteristics. [10]

Toxins that affect sodium channels, especially those with lengthy chains, fall under two main categories: α and β toxins. [9] The polypeptides consist of 61 to 76 amino acids and are held together securely by four disulfide bonds. [11] These molecules can be grouped into two main categories, determined by their binding characteristics and how they regulate channel gating. [12]

Scorpion α-Toxins:

The α-toxins engage with site three in vertebrates on the channel's extracellular surface. [13] The working of these toxins is independent of the environment of the membrane, and they cause both muscles and neurons to extend their action potential. [14] They influence the membrane's electrical state and affect how quickly the sodium

channels can inactivate. [15] The three primary categories of α-toxins are anti-insect α-toxins, α-like variants, and classical α-toxins.

Scorpion β-Toxins

β-toxins are derived from scorpions native to America and target a specific receptor site four on vertebrate sodium (Na+) channels. When they bind to these channels, they cause the membrane potential to become more negative. [16] Research has explored how these toxins affect sodium levels, revealing that they can significantly alter cellular activity. However, it is essential to note that not all sodium channel toxins (NaTxs) neatly fit into the two main categories. For instance, *Centruroides noxius* species-derived Cn12 and *Tityus serrulatus* venom-derived Ts2 share structural similarities with β-type toxins but behave more like α-type toxins. [17][18] *Androctonus australis Hector* produces the toxin AaHIT4, which has alpha- and beta-type properties. [19] We can classify β-toxins into different sub-groups. The first group comprises classical β-toxins specifically targeting mammals, while another group can affect mammals and insects. [20] There are additional types of NaTxs, such as those originating from the New World, which mainly affect insects and crustaceans. Additionally, some toxins are known to be competing for binding sites on excitable receptors in insects and in both alpha- and beta-type mammal toxins. [21][11][22][23]

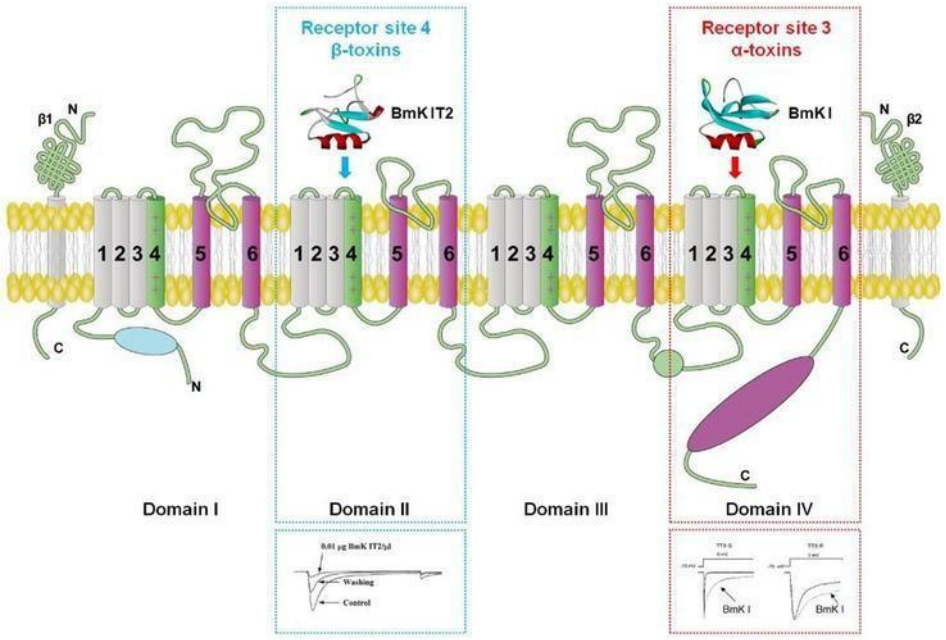


Fig. 3 A visual depiction of sodium channel alpha and beta subunits. [24]

Another set of peptides in the short scorpion toxin family is characterised by three or four disulfide bridges, ranging from 23 to 64 amino acids in length. The primary function is to block the potassium ion channel; these are called potassium channel toxins (KTxs). [25] Potassium channels are crucial for various physiological processes, including regulating electrical activity in neurons and the heart, muscle contractions, releasing neurotransmitters, hormone secretion, and activating immune cells. [26][27] KTxs could be categorised into several groups—α, β, γ, κ, δ, λ, and ε—based on their sequences and the arrangement of cysteine pairs. The largest subgroup is the α-KTx group, which includes 23 to 42 residues of 3- or 4-disulfide-bridged long peptides. The β-KTx category includes extended peptides ranging from 50 to 75 amino acids. On the other hand, the γ-KTx category is found in the genera *Mesobuthus, Centruroides,* and *Buthus,* with their toxins primarily functioning as inhibitors of the human Ether-à-go-go Related Gene (hERG) channels.[28] The cell cycle and the development of different cancers are influenced by the HERG channels. This suggests that specific blockers targeting these channels could reduce tumour cell proliferation. [29] Unlike the typical α/β structure found in the α, β, and γ-KTx subfamilies, the κ-KTx group is characterised by two short, parallel α-helices that are joined by a β-turn and reinforced by two disulfide links. Even though κ-KTx toxins have varying structures, they still engage with potassium ion channels like the α-KTx group. A lysine and a hydrophobic amino acid, typically tyrosine or phenylalanine, interact in this way, which is often readily accessible on the flat surfaces of the toxin, allowing for easy interaction with the channel. [30]

Calcins represent a modest yet expanding group of scorpion venom peptides that regulate calcium channels. Some well-known members include urocalcin, maurocalcin, imperacalcin, hadrucalcin, opicalcin, hemicalcin, and vejocalcin. [31] Usually, most calcins have 33 amino acids, but it is observed that in hadrucalcin, due to the extended N-terminus, it has a total of 35 amino acids. These peptides align precisely due to their conserved structure having six cysteine residues with uniform spacing between them. Specific disulfide bridges have been shown in maurocalcin and imperacalcin, highlighting arrangements that contribute to a unique feature known as the inhibitor cysteine knot (ICK) motif. [32] Calcins have varied sizes, resulting in different molecular masses, where imperacalcin is the lightest with about 3758.5 daltons, and hadrucalcin is the heaviest at around 4190.8 daltons. The volume of these molecules also varies, from 2,692.7 Å^3 for vejocalcin to 3,001.9 Å^3 for hadrucalcin. When we look at their amino acid composition, there are plenty of positively charged residues like lysine and arginine, which range from 9 (27% in vejocalcin) to 13 (39% in urocalcin). On the other hand, negatively charged residues such as aspartate and glutamate are less common, appearing only 3 (9% in vejocalcin) to 5 (14% in hadrucalcin) times. This imbalance between positive and negative charges at pH 7.0, calcins provide a net positive charge and have a high isoelectric point (pI = 9.3–10.1) ranging from 5.8 (in vejocalcin) to 8.7 (in urocalcin). [17] Calcins also share a high sequence similarity, with over 78% identity, and feature the ICK motif reinforced by three disulfide bonds. [33] The main function of calcins is that they act as an agonist to ryanodine receptors (RyRs) located in the endo/sarcoplasmic reticulum. Ryanodine receptors are essential for releasing calcium from the internal stores and are thus crucial for muscle function, especially during contraction. Generally, calcins in RyR channels can cause extended subconductance states, increasing calcium levels inside the cell and muscle contractile paralysis. [31]

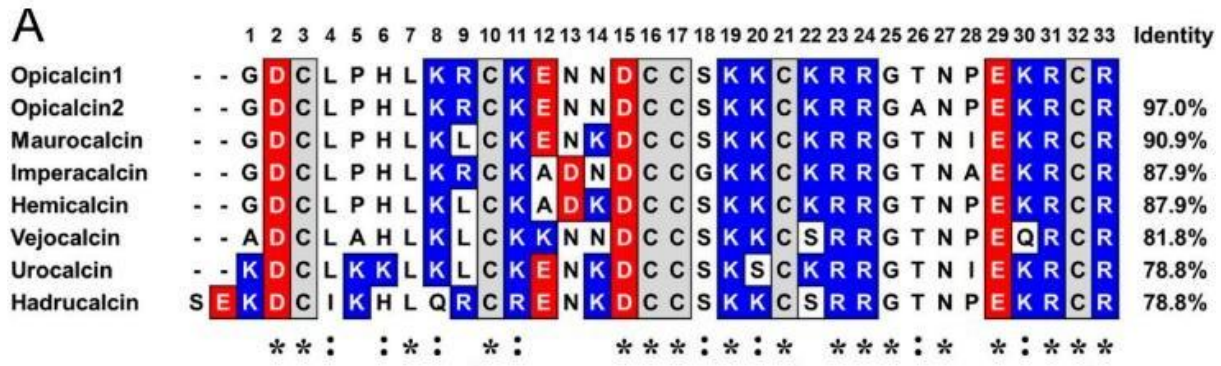


Fig 4. The Clustal Omega tool aligned the sequences of the eight calcins discovered thus far, using opicalcin1 as the reference. On the right are the identity values that correlate to opicalcin1. This alignment highlights negatively charged residues like aspartic acid (D) and glutamic acid (E) in red and positively charged residues like lysine (K) and arginine (R) in blue. Furthermore, grey symbolises cysteine (C), which creates disulfide bonds. Asterisks indicate columns with identical residues, whereas the colons below indicate columns with only one different residue. [34]

Researchers have recently discovered different enzymes in scorpion species' venom, including hyaluronidases, phospholipases, and metalloproteases. [78] Researchers have identified distinct types of hyaluronidases in several scorpion families, like *Bothriuridae*, *Buthidae* and *Urodacidae*. [35] These enzymes enhance the venom's toxicity by breaking down the connective tissues and extracellular matrix around blood vessels at the site of the sting. [36] Other toxins also spread more efficiently throughout the body due to this disruption. Recent studies have shown that hyaluronidases are crucial for how venom travels from the bloodstream to target organs. [37] Moreover, neutralising these enzymes might be a functional first-aid approach for treating scorpion stings. Phospholipases, another group of enzymes, are powerful hemolytic agents; breaking the phospholipids is known to damage the cell membrane, further causing bleeding and tissue damage. Alongside these, the venom of *T. discrepans* [38] and *Hemiscorpius lepturus* [39] contains serine proteases and metalloproteases.

These proteases are thought to be essential for activating toxin precursors through modifications that occur after the proteins are formed. [40]

Additionally, short-chain neurotoxins (SCNs) act as chloride channel blockers. These neurotoxins are about 30-40 residues long, and feature four disulfide bridges labelled C1-C4, C2-C6, C3-C7, and C5-C8.

There has been an incredible rise in antimicrobial peptides (AMPs) over the past ten years. It shows that a modest collection of 50 peptides rose to about 200 unique AMPs, as catalogued in the UniProt database. Research is being conducted to isolate and characterise the peptides and explore their potential therapeutic applications. [41] AMPs can disrupt cell membranes, alter ion permeability, and affect cellular functions by interacting with significant cellular pathways, including intracellular signalling and G-proteins. [41] These AMPs are typically short, positively charged, and amphipathic in scorpions. They can be categorised into three structural groups: 1. Peptides with cysteine residues connected by disulfide bridges are included in the first group. 2. Peptides without cysteine that have an amphipathic α-helix shape comprise the second group. 3. Proline and glycine residues are abundant in the third group.

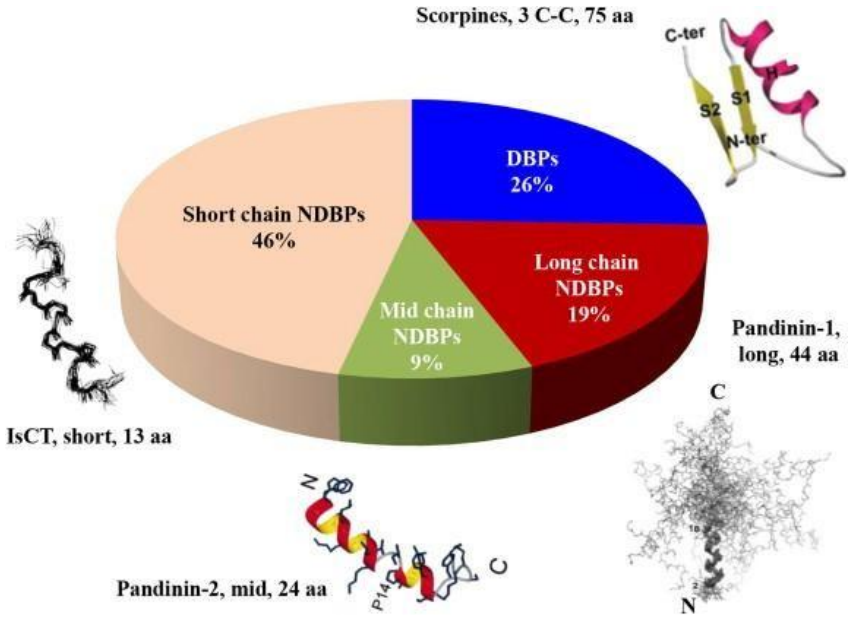


Fig. 5 Antimicrobial Peptides from Scorpion Venom [42]

Cysteine-rich antimicrobial peptides (AMPs) typically have three to four disulfide bonds. For instance, Scorpine, the derived peptide from the species of scorpion *Pandinus imperator*, constitutes only 1.4% of the venom and has three disulfide bridges. The mature scorpine has 75 amino acids (aa) encoded by the internal sequence, concluding with a stop codon at position 76. Two significant nucleotide segments are also present: one located at the 5' terminal that probably codes for a signal peptide made up of 19 amino acids (aa), and another found in the 3' untranslated region that suggests a location for adenylation. [43]

More members of the scorpine family have been discovered in the last few years. Opiscorpine comes from *Opistophthalmus carinatus* [44][45] and Heteroscorpine-1 (HS-1), which is derived from the scorpion species venom *Heterometrus laoticus*.[46]

The precursor for Opiscorpine consists of 95 amino acids (aa). It comprises a signal peptide composed of 19 amino acids (aa) located at the beginning, followed by a mature peptide segment of 76 residues. This family shares roughly 70% similarity at the level of the amino acid sequence of P. imperator's scorpine, with a specific deletion/insertion mutation identified at position 55. [45]

Heteroscorpine-1 also comprises 95 amino acids (aa), including a 19-residue signal sequence at the N-terminus. It features six cysteine residues, which indicate sufficient disulfide bond formations within the molecule. Additionally, there's an intron of 1,073 nucleotides surrounded by two exons. On comparatively aligning sequence of amino acids in the HS-1 peptide with Panscorpine and Opiscorpine, it shows us a similarity of approximately 82% and 78%, respectively. In addition, it shows a 40% similarity to several potassium channel-blocking peptides, highlighting its close evolutionary relationship with other scorpine peptides on phylogenetic trees. This is affirmative evidence for HS-1 being a part of the scorpine family of peptides, which possesses potassium channel-blocking and defensin activities. [46][44] The scorpine-like peptides were initially termed 'orphan peptides' since

they exhibited bifunctionality due to their contrasting pharmacological activity. The N-terminal region of scorpine-like peptides possesses a significant activity for cytolysis (cell-lysing) or antimicrobial activity. [29][30] Conversely, the C-terminal segment possesses the ability to block potassium channels and is compactly folded through three disulfide bonds, demonstrating a "cysteine-stabilized α/β motif" (CS-α/β). [47][48]

Another peptide, BmTXKS2, which has paved its way as a complete-length complementary DNA (cDNA) clone from venom-filled glands of the Chinese scorpion, *Buthus martensii Karsch.* BmTXKS2 is estimated to consist of 39 residues, including six cysteines, and is reminiscent of hemolymph defensins. However, to this date, research has not isolated its native or recombinant form; there are no reports on its activity. [37,38]

Scorpion NDBPs are fascinating molecules that come in various lengths, typically ranging from 13 amino acid residues to 56 amino acid residues. They exhibit considerable sequence diversity, adding to their complexity. Our understanding of their structures primarily comes from studies using Circular Dichroism and bioinformatics tools that help predict their secondary structures. Most of these peptides are rich in alpha-helical formations. Interestingly, they can be categorised based on how these helical regions are arranged into three principal families. The first family comprises peptides featuring a single alpha-helical domain flanked by two random coil segments at the N- and C-termini. [39] Examples of this structure include Mauriporin, IsCT2, AamAP1, Meucin-24, BmKn2, Im-1, Pandinin 2, AamAP2, HsAP, IsCT, BmKb1. [49][50][51] [52] [53] [54] [55] [56] [57] In the second family, the peptides contain two alpha-helical segments separated by a random coil. Well-known members of this group are Parabutoporin, Opistoporin 1, Hadrurin, BmKbpp, Pandinin 1. [58] [59][60][50]The third family is less common; it includes fully helical peptides throughout. Imcroporin and StCT2 are examples of this fully helical structure. [61][62]

One particularly interesting peptide is Hadrurin, derived from the *Hoffmannihadrurus aztecus* scorpion venom. This particular peptide comprises 41 amino acids (aa), accounting for 1.7% of the total venom content. Notably, it has a high proportion of basic amino acids, including a triplet sequence of Lys-Arg-Lys. Uniquely, Hadrurin does not contain cysteine residues, typically prevalent in scorpion venoms. [63]

Another peptide of interest is Pandinin-1, which is found in the venom of the scorpion species *Pandinus imperator* and consists of 44 amino acids (aa). Its counterpart, the smaller helical peptide, is called pandinin-2, a peptide comprising 24 amino acids. [64][51]

Opistoporin 1, a peptide with antimicrobial properties, is extracted from the venom of the South African venomous scorpion *Opistophthalmus carinatus.* It contains 44 amino acids (aa) and features both charged and neutral residues, resulting at neutral pH; this results in a net positive charge of +4. [65]{Twelve charged residues were present in the peptides: three glutamate, eight lysine, and one aspartate.)}. In reference to the genus of scorpions from which they were extracted, the peptides were designated opistoporin 1 (amino acid 34L) and 2 (amino acid 34F). In comparison, Parabutoporin has an even higher charge of +7. [66] These peptides, like many others, do not have cysteine residues. A random coiled region (WNSEP) divides the two a-helical domains (residues 3–14 and 20–39) in both opistoporins. Hadrurin and pandinin have also been predicted to have this structure. [59]

IsCT is a novel, short, and remarkable natural cytotoxic peptide comprising 13 amino acids (aa) isolated from *Opisthacanthus madagascariensis* scorpion venom. It is noted for its high antimicrobial activity and could be valuable for research related to lipid-peptide interactions. [51]

Meucin-24 and Meucin-25 were also discovered using the complementary DNA (cDNA) library extracted from the venom gland of the scorption *Mesobuthus eupeus*. The precursor for these peptides consists of 46 amino acids (aa), which is a mature peptide sequence consisting of 24 amino acids (aa) with a predicted isoelectric point of 10.17 and a signal peptide consisting of 22 amino acids (aa). Meucin-24 shows similarities to known cationic antimicrobial peptides (magainin 1 and 2) found in frog skin. [67][68]

Meucin-24 is a shortened product containing the mature MeuTXKb3's five amino-terminal residues and the propeptide. compared to the precursor structure of BmTXKb-related peptides. Meucin25 is a 25-residue mature carboxyl-terminal peptide and a 31-residue amino-terminal signal peptide comprising the 56 amino acid precursor for clone BeL-170. Secondary structural prediction indicates meucin-25 would exist in a combined β-sheet and a-helix structure.[68]

The SignalP 3.0 tool http://www.cbs.dtu.dk /services/SignalP/ was used to identify the supposed 22-residue signal peptide found in imcroporin, which was extracted from the scorpion *Isometrus maculates* cDNA library. [69] followed by a mature peptide that is believed to consist of 17 residues and an unusual acidic propeptide located at the C terminus. Gly-Arg-Arg, a preserved signal for post-translational modification, appears at positions 40 to 42 of the 35-residue propeptide. In the past, when a propeptide with a particular processing signal was removed, it would form a mature peptide with an amidated C-terminus.[62]

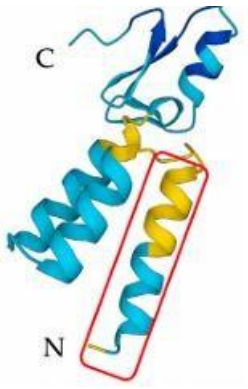
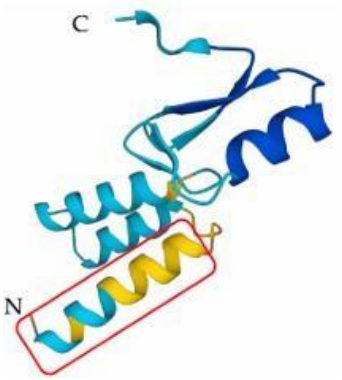
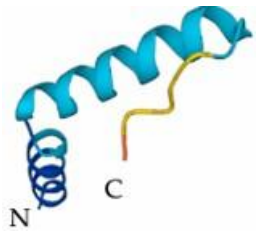
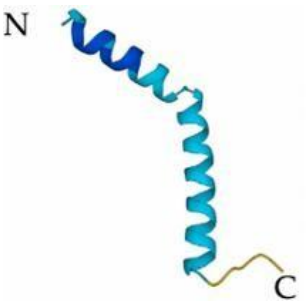
The self-optimized prediction method (SOPM) was used to forecast the mature peptide's secondary structure. [69] It is anticipated that the mature peptide of imcroporin will have a 100% -helix secondary structure. [70] A line separates the helical wheel into two halves. The hydrophilic face and the hydrophobic face are the two components. Imcroporin appears to be a promising dipolar compound based on this study.

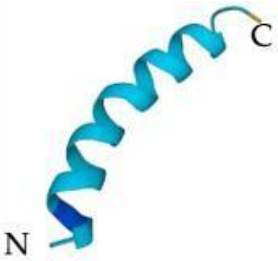
Table 1. AMPs, or antimicrobial peptides, are extracted from various scorpion species' venom and compiled here.

Name (UniProtKB)	**Sequence**	**Length(A)/molecular weight (Da)**	**Activity Preferences**	**Scorpion species (Family)**	**Reference**
AMPs containing cysteine residue					
Scorpine or Panscorpine	GWINEEKIQKKIDERMGNTVLGG MAKAIVHKMAKNEFQCMANMD MLGNCEKHCQT SGEKGYCHGTKCKCGTPLSY	75/8350 ;3DSBs	Against bacteria parasite (*P.berghei, B.subtilis* & *K.pneumonia*).	*P. imperator (Scorpionidae)*	[43]
Heteroscorpine	GWINEEKIQKKIDEKIGNNILGGM AKAV VHKLAKGEFQCVANIDTMGNCET HCQK TSGEKGFCHGTKCKCGKPLSY	76/8293 ; 3DSBs	Against bacteria (*B.subtilis, P.aeruginosa* & *K.pneumonia*).	*H. laoticus (Scorpionidae)*	[46]
Opiscorpine-1	KWFNEKSIQNKIDEKIGKNFLGGM AKAV VHKLAKNEFMCVANVDMTKSCD THCQ KASGEKGYCHGTKCKCGVPLSY	76/8428 ;3DSBs	Against the bacteria *E.coli, P.aeruginosa* & yeasts *F.oxysporum, F.culmorum*	*O.carinatus (Scorpionidae)*	[45]
Opiscorpine-2	KWLNEKSIQNKIDEKIGKNFLGG MAKAV VHKLAKNEFMCMANMDPTGSCE THCQK ASGEKGYCHGTKCKCGVPLSY	76/8367 ; 3DSBs	Against bacteria & yeasts.	*O.carinatus (Scorpionidae)*	[45]
Opiscorpine-3	KWLNEKSIQNKIDEKIGKNFLGG MAKAV VHKLAKNEFMCVANVDMTKSCD THCQK ASGEKGYCHGTKCKCGVPLSY	76/8394 ; 3DSBs	Against bacteria & yeasts.	*O.carinatus (Scorpionidae)*	[45]
Opiscorpine-4	KWLNEKSIQNKIDEKIGKNFLGG MAKAV VHKLAKNEFMCVANIDMTKSCDT HCQK ASGEKGYCHGTKCKCGVPLSY	76/8408 ; 3DSBs	Against bacteria & yeasts.	*O.carinatus (Scorpionidae)*	[45]
2. Non-cysteine-containing AMPs-Long-chain					

Opistoporin-1	GKVWDWIKSTAKKLWNSEPVKELKNTA LNAAKNLVAEKIGATPS	44/4836	Broad Spectrum, against Gram-positive & Gram-negative bacteria; against fungi.	*O.carinatus* (Scorpionidae)	[59]
Opistoporin-2	GKVWDWIKSTAKKLWNSEPVKELKNTA LNAAKNFVAEKIGATPS	44/4870	Broad Spectrum, against Gram-positive & Gram-negative bacteria; against fungi.	*O.carinatus (Scorpionidae)*	[59]
Hadrurin	GILDTIKSIASKVWNSKTVQDLKRKGINW VANKLGVSPQAA	41/4436	Against *S.marcescens, S. typhimurium, P.aeruginosa, K.pneumoniae, & E.coli*	*H.aztecus (Iuroidea)*	[58]
Pandinin-1	GKVWDWIKSAAKKIWSSEPVSQLKGQV LNAAKNYVAEKIGATPT	44/4800	Against Gram-positive bacteria *B.subtilis, E. faecalis, S.epidermidis, & S.aureus.*	*P. imperator (Scorpionidae)*	[51]
3. Non-cysteine-containing AMPs-Intermediate chain					
Meucin-24	GRGREFMSNLKEKLSGVKEKMKNS	24/2753.95	Against P. berghei ookinetes	*M. eupeus (Buthidae)*	[68]
Meucin-25	VKLIQIRIWIQYVTVLQMFSMKTKQ	25/3095.56	Against P. berghei ookinetes	*M. eupeus (Buthidae)*	[68]
Pandinin-2	FWGALAKGALKLIPSLFSSFSKKD	24/2612	Against Gram-positive bacteria *B.subtilis, S.epidermidis, E. faecalis* and *S.aureus*	*P. imperator (Scorpionidae)*	[51]
4. Non-cysteine-containing AMPs-Short-chain					
IsCT	ILGKIWEGIKSLF-NH2	13/1502	Against Gram-positive & Gram-negative bacteria	*O.madagascariensis (Hemiscorpiidae)*	[63]
Imcroporin	FFSLLPSLIGGLVSAIK-NH2	17/1761	Against Gram-positive bacteria *B. thuringiensis M. luteus, B.subtilis* and *S.aureus*	*I.maculatus (Buthidae)*	[62]

Table 2. Selected antimicrobial peptides (AMPs) derived from scorpion venom, their three-dimensional structures acquired from the UniProt database, and their mechanisms of action against different bacterial strains. [71]

AMP	Mechanism of Action	Structure
1. Cysteine containing AMPs		
Scorpine	Hydrophobic interactions and cell penetration cause membrane rupture.	
Heteroscorpine-1	Blistering that appears on the membrane.	
2. Non-cysteine-containing AMPs		
Hardrurin	Electrostatic forces and hydrophobic interactions are used to lyse acidic liposomes and zwitterionic phospholipids.	
Pandinin-1	Disruption of membranes and the creation of pores.	

Pandinin-2	Interaction with the breakdown of lipid membranes and the creation of pores.	

Structural activity relationship of AMPs

Peptide Sequence and Structure

Antimicrobial peptides (AMPs) derived from scorpions are typically short sequences comprised of 10 to 25 amino acid residues. Their compact sizes not only simplify chemical synthesis, making them more cost-effective [72] but also make them suitable for large-scale production. Remarkably, research using artificial intelligence has shown that some exceptionally short AMPs, containing just nine residues, can have minimum inhibitory concentrations (MIC) below one µM [73]. C-terminal amidation is a critical modification for these peptides, which protects them from breakdown and enhances their antimicrobial activity by stabilising interactions with cell membranes [74,75].

Hydrophobicity

Hydrophobicity is a crucial factor in how AMPs interact with the cytoplasmic membrane. Increased hydrophobicity typically boosts antimicrobial activity as peptides engage more effectively with membranes. However, this can also raise the risk of toxicity to mammalian cells. [76]

Hydrophobic Moment

There is a distinct relationship between a peptide's hydrophobic moment and how effectively it can act against microbes. A higher hydrophobic moment usually means stronger antimicrobial action. This metric reflects a peptide's hydrophobic characteristics across various rotational angles [50], [77,78]. For example, researchers found that five analogue peptides derived from the AMP BmKn1 of *Buthus martensii* exhibited enhanced antimicrobial activity as their hydrophobic moments increased [79].

Net Charge

Many AMPs share a cationic nature, significantly influencing their interaction with microbes. The overall net charge from all ionisable groups affects how these peptides bind to bacterial membranes. For instance, TsAP-1, a peptide from *Tityus serrulatus* with a charge of +2, was compared to its analogue TsAP-S1, which has a charge of +6. The study revealed that TsAP-S1 was significantly more potent—showing 48 times greater effectiveness against *Staphylococcus aureus*, 32 times against *Escherichia coli*, and 64 times against *Candida albicans.* [80]

Polar and Hydrophobic Angles

The polar angle (θp) and hydrophobic angle (θh) of peptides influence their behaviour upon interacting with membranes. It is more likely that peptides with low polarity and high hydrophobic angles may cause membrane holes. In contrast, those with balanced polar and nonpolar characteristics (amphipathic) usually attach parallel to the membrane, with lipids and hydrophobic regions interacting. [81] Interestingly, AMPs with a higher leucine content can enter the lipid bilayer more deeply, and increasing the polar angle tends to reduce hemolytic activity, lowering the risk of damage to host cells.

Scorpion Venom: A Multifaceted Ally in Medicine

Scorpion venom is a fascinating brew of substances produced in specialised glands of these creatures. When introduced into their prey or adversaries through a stinger, venom immobilises or paralyses them [82]. The specific amount of venom varies by species and the intensity of the sting. Some notable venomous scorpions with potential

pharmaceutical benefits include *Tityus discrepans, Buthus martensii, Pandinus imperator, Chaerilustricostatus, Mesobuthus eupeus, Leirus quinnquestriatus,* and *Heterometrus bengalensis* [82].

Historically, scorpion venom has been utilised to treat various ailments. For instance, scorpion oil has been applied to fight against tumour cells, infections, and inflammation. Notably, when a person is envenomated, their autonomic nervous system is affected, resulting in increased insulin levels and the release of hormones like glucagon, cortisol, and angiotensin II [83].

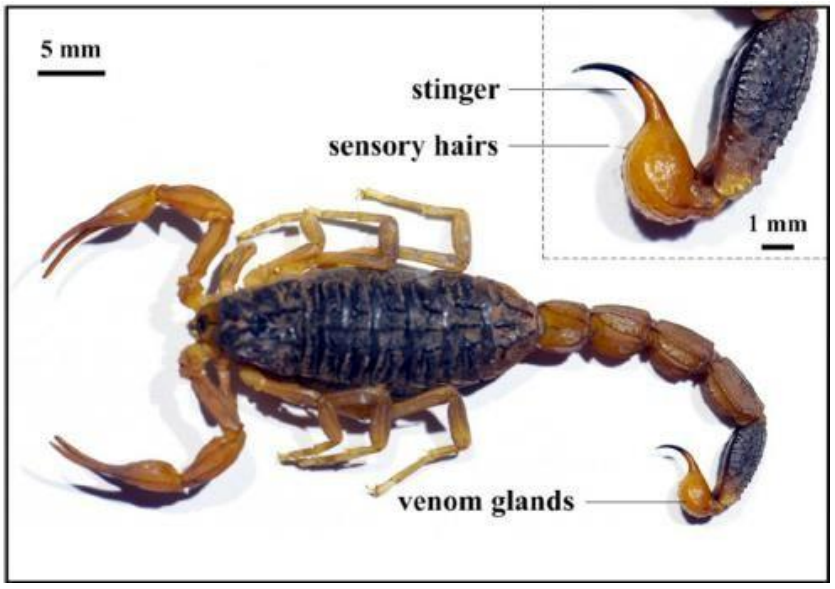


Fig 6 Aerial perspective of the scorpion species *Buthus martensii Karsch.* Featured in the corner is a zoomed-in image highlighting its stinger. [84]

Advancements in Antivenom Research

Scorpions present a notable threat to both humans and animals across various regions of the globe. To combat the effects of scorpion stings more effectively, researchers have introduced nanobodies (Nbs). NbAahIF12 and NbAahII10 have shown promising ability to neutralise the venom from the *Androctonus australis* species.

Preclinical studies indicate that a bispecific nanobody construct known as NbF12-10 is the most effective treatment for scorpion envenomation, surpassing traditional Fab′2-based therapies in its protective abilities. Research employing in vivo imaging in rodents has allowed scientists to examine the pharmacokinetics and distribution of 99mTc-labeled nanobodies, comparing them to the Fab′2 product [85].

In addition, echocardiographic research conducted on rats has demonstrated that NbF12-10 can successfully mitigate the hemodynamic disruptions induced by a fatal dose of venom. Notably, NbF12-10 can restore blood pressure and heart rate to normal, even when administered later. [86] Histological analyses indicate that this nanobody protects the hearts and lungs of treated mice from damage during envenomation. This preclinical research suggests that NbF12-10 can prevent the life-threatening effects of *Androctonus* venom, showing potential to replace traditional treatments as a novel immunotherapeutic approach in cases of scorpion stings. Further clinical trials with larger animal groups are needed to confirm the full protective capabilities of NbF12-10 [85].

Scorpion Venom in Cancer Therapy

Scorpions have venom glands that produce a diverse array of substances. This includes non-protein elements like lipids, inorganic salts, water, free amino acids, and important proteins such as enzymes and peptides. These components play a crucial role in helping scorpions defend themselves and catch their prey. Recent studies and experimental research have shown that raw scorpion venom and specific isolated proteins and peptides can interfere with critical features of cancer, proving to be effective in laboratory settings and living organisms. [87]

Research has explored the effectiveness of scorpion venoms in treating a range of cancers, including glioma, leukaemia, neuroblastoma and lymphoma, and lung, breast, liver, pancreatic, and prostate cancers. [88] The anticancer properties of scorpion venom appear to be linked to certain refined toxins. These promising findings underscore the potential for utilising scorpion venom and its active components in cancer treatments. [87]

Table 3: Promising anticancer venom/toxins from scorpion

*Characteristics of cancer include: 1. Uncontrolled cell growth, 2. Resistance to programmed cell death, 3. Ongoing development of new blood vessels, and 4. Infiltration of surrounding tissues and spread to other areas. •The symbol represents the cell lines examined in xenograft tumour studies.[87]

Species	Compound	Target/Mechanism	Effect on cancer Hallmarks*	*In-vitro* cancer cell lines and *in-vitro* tumour models
Buthus martensii Karsch (BmK)	Whole Venom	Increases PTEN and p27; inhibits cyclin D1; stops the cell cycle on G0/G1; and upregulates caspase 3.	1, 2	Human hepatoma (SMMC7721); Human lymphoma (Raji and Jurkat); Human breast cancer (MCF-7); Human glioma (U251-MG)
	PESV	Decreases PI3K and Akt; raises PTEN; stops the cell cycle at G0 or G1; lowers mTOR; lowers VEGF; reduces the density of microvessels.	1, 2, 3	Murine hepatoma (H2-2); Human leukemia (K562); Human lung (A549)
	BmKn-2	Increases p53 and BAX; reduces Bcl-2; and increases caspase-3, 7, and 9.	2	Human mouth epidermoid carcinoma (KB); Human oral squamous carcinoma (HSC-4)
	LMWSVP	Reduces Bcl-2 and increases caspase-3.	2	Human hepatoma (SMMC7721)
	GST-BmKCT	Inhibits the Cl-channel and lowers MMP-2	1,4	Rat glioma (C6)
	Ad-BmKCT	Inhibits the Cl-channel and lowers MMP-2	1, 4	Rat glioma (C6)•
	rAGAP	Reduce pAkt, VEGF, and MMP-9; inhibit migration; stop the cell cycle on G1; suppress CDK2, CDK6, and pRb.	1, 3, 4	Rat glioma (C6) Human; anaplastic astrocytoma (SHG-44)
	BmKKx2		1, 2	

	TM-601	It inhibits differentiation, suppresses proliferation, blocks K2+ channels, and encourages differentiation-dependent death. Blocks Cl^- channel	4	Human myelogenous leukemic (K562) Human glioblastoma (U87); Rat glioma (F98)
Androctonus amoreuxi	Whole venom	Reduces VEGF, increases caspase-3, causes DNA fragmentation, and inhibits colony formation and cell motility.	2, 3, 4	Human breast cancer (MCF-7); Ehrlich ascites and solid tumors
Androctonus crassicauda	Whole venom Acra3	Depolarises the mitochondrial membrane, increases caspase-3, stops the cell cycle in S-phase, and reduces colony formation and cell motility. ----------	1, 2, 4 2	Human breast cancer (MCF-7); Human breast carcinoma (MDA-MB-231); Human neuroblastoma (SH-SYSY);Human ileocecal adenocarcinoma (HCT-8); Human colorectal carcinoma (HCT-116) Mouse brain tumour (BC3H1)
Heterometrus bengalensis Koch	Whole venom Bengalin	Stops the cell cycle; causes chromatin condensation, membrane blabbing, and DNA deterioration Causes DNA breakage, reduces telomerase activity, reduces the potential of the mitochondrial membrane, and activates caspase-3, 9.	1, 2 2	Human leukemic (U937, K562) Human leukemic (U937, K562)
Tityus discrepans	Whole venom, neopladine one and neopladine 2	Cause DNA fragmentation and the expression of FasL	2	Human breast (SKBR3)
Odontobu-thus doriae	Whole venom	Boosts caspase-3 and causes mitochondria to depolarise.	1, 2	Human breast (MCF-7); Human neuroblastoma (SH-SYSY)
Rhopalurus junceus	Whole venom	Reduces bcl-2 mRNA; increases p53 and caspases 3, 8, and 9;	2	Human breast (MDA-MB-213, MDA-MB-468); Human lung (A549, NCI-H292)

		and causes chromatin condensation.		

Exploring the Anti-Microbial Potential of Scorpion Venom

1. Antibacterial Peptides in Scorpion Venom

Innovative research areas are emerging to understand how scorpion venoms can be used medicinally, especially for their antibacterial capabilities. Many different types of antimicrobial peptides (AMPs) have been established in scorpion venom, which can have therapeutic applications. [89] For example, hadrurin, a peptide found in the venom of the *Hadrurus gertschi* species, Scorpine, and some Pandinins from the *Pandinus imperator* were some of the earliest scorpion venom AMPs to be studied. [43,58] IsCTs from *Opisthacanthus madagascariensis* and some Opisthoporins from *Opistophtalmus carinatus* have also been described. [59,63] Nevertheless, one major limitation of applying such AMPs in clinical practice is their relatively high toxicity towards eukaryotic cells. In most cases, the dose effective in treating infections is just a few fold lower than that harmful to human cells. For instance, hadrurin, which is the first antibacterial peptide isolated from scorpions, is found to be very cytotoxic to human blood cells at 30μM but can be used to treat various Gram-positive and Gram-negative bacterial infections at 10-50μM range. [58] This overlapping range of toxicity and effectiveness has led researchers to consider these compounds primarily for topical applications initially. [90] Despite these concerns, the therapeutic potential of scorpion AMPs remains promising. The hemolysis activities of the remaining AMPs discovered in scorpion venoms were determined to be highly hemotoxic. However, their usefulness in the hunt for substitute antibiotics is still valid. Encouragingly, research has shown that it is possible to enhance the antibacterial efficacy of these peptides while simultaneously reducing their toxicity to human cells. This dual approach could pave the way for more viable antibiotic alternatives, addressing the pressing requirement for novel treatments due to the growing problem of antibiotic resistance.

2. Antifungal Peptides in Scorpion Venom:

Researchers have been exploring the antifungal properties of various NDBPs found in scorpion venom, discovering several effective ways to fight important clinical pathogens. For instance, both parabutoporin and optostoporin one can inhibit the growth of the yeast *Saccharomyces cerevisiae* by 50% effective at their concentration of just two μM [59,91] Furthermore, pandinin-2 exhibits a minimum inhibitory concentration (MIC) of 19.1 μM, effectively halting the growth of Candida albicans [51,92].

Meucin 18 has been shown to have lethal doses of 8.3 μM, 25.1 μM, and 10.9 μM against *Aspergillus fumigatus, C. albicans,* and *S. cerevisiae,* respectively [93]. Additionally, at an MIC of 20 μg/ml, ctriporin proved to be effective against Candida albicans. The growth of *Candida tropicalis* was inhibited by pantinin-1, -2, and -3 at MICs of 16 μM, 16 μM, and 17 μM, respectively [90]. Notably, the AamAP-S1 peptide, engineered from *Androctonus amoreuxi*, showed over ten times the potency of its parent peptide, AmAP1, against *Candida albicans* with a MIC of just five μM.

3. Antimalarial Peptides in Scorpion Venom:

The journey into antimalarial peptides began with scorpine, a three-disulfide-bridged peptide derived from the venom of the scorpion species *Pandinus imperator* [43]. This peptide was shown to significantly inhibit key stages in the growth of *Plasmodium berghei*, a mouse model for malaria that mimics the life cycle of *Plasmodium falciparum* after infection from a mosquito bite. Scorpine effectively inhibited the growth and development of *P. berghei* during the ookinete stage (with a median effective dose, ED50, of 0.7 μM) and the gamete stage (ED50 of 10 μM).

Notably, scorpine has been at the forefront of innovative antimalarial strategies. The transgenic scorpine-expressing fungus *Metathizium anisopliae* prevented mosquitoes infected with *Plasmodium* from further spreading the disease [94].

Furthermore, there have been remarkable findings on NDBPs which are antimalarial such as meucin-24 and meucin-25 isolated from the venom gland of scorpion *Mesobuthus eupeus*. These two peptide sequences are not

alike in structure, yet both inhibited the in vitro growth of *P. berghei* ookinetes within the concentration range of 10 to 20 μM. Moreover, the peptides were able to kill the intraerythrocytic stages of *P. falciparum*, reducing the parasite density to a great extent within 48 hours and clearing the erythrocytes from the parasite within 72 hours. [95]

What is particularly promising is that neither meucin-24 nor meucin-25 exerted any toxic effects on the bacteria and fungi at the tested concentrations, demonstrating selectivity toward *Plasmodium.* Moreover, no hemolysis was observed on murine erythrocytes, even at high doses of 100 μM. [96] Therefore, meucin-24 and meucin-25 present excellent prospects for antimalarial treatments. Similarly, AMPs like vejovine and hadrurin have exhibited antibacterial activity, with further tests showing that some modified variants reduced *P. berghei* ookinete production by 40% at a dose of 5 μM, and this inhibition doubled at 25 μM [97].

4. Antiviral peptides in scorpion venom:

Recent research has revealed that specific peptides from scorpion venom hold enormous promise as antiviral drugs. [98] One of the first peptides to be identified is Hp1090, sourced from the scorpion *Heterometrus petersii* venom. [99] Hp1090 has shown impressive effectiveness, with an IC50 of 5 μM, in preventing hepatitis C virus (HCV) infection in laboratory settings. Remarkably, it outperformed the commonly used antiviral drug IFN-α, inhibiting HCV RNA amplification in Huh7.5.1 cells at around 13 μM. This peptide functions by engaging directly with viral particles, causing a disruption in their phospholipid membranes, thereby blocking the virus from initiating an infection [100].

The potential of Hp1090 as a candidate for hepatitis C treatment is exciting. Another interesting peptide, mucroporin-M1, initially recognised for its antibacterial properties, has also demonstrated antiviral activity against several viruses, including the H5N1 Influenza A virus (EC50 of 1.03 μM), the SARS coronavirus (EC50 of 7.12μM), and the measles virus (EC50 of 3.52 μM). Ironically, the original version of mucroporin did not show effectiveness against these viruses [101]. This finding suggests that cationic peptides sourced from scorpion venom may hold great potential for creating flexible antiviral treatments.

Another noteworthy peptide, Kn2-7, is a modified version of previous scorpion venom peptides and has exhibited strong activity against HIV-1, with an EC50 value of just 1.65 μM and minimal toxicity to host cells. Regardless of their tropism traits, Kn2-7 has shown the capacity to block all 13 strains from a typical panel of HIV-1 subtype B pseudotyped viruses. It nearly completely blocked viral replication in cell-free environments and replicating HIV-1 associated with host cells at approximately nine μM. This ability to swiftly damage viral membranes reinforces the potential of AVPs; the activity of Kn2-7 against HIV-1 is linked to its direct interaction with the HIV-1 envelope [102].

In a very interesting recent development, it has been reported that a peptide named Ctry2459, originating from the scorpion *Chaerilus tryznai*, was able to prevent HCV infection by trying to target and deactivate infectious viral particles. Still, it was said that its effectiveness was hampered as it exhibited low levels of bioavailability within the infected cells, which failed to suppress ongoing infections. Towards this, two additional peptides (Ctry2459-H2 and Ctry2459-H3) were designed to increase its cellular distribution and uptake compared to the original Ctry2459 one. [103] While showing comparable cytotoxicity and hemolytic activity to their parent peptide, these modified peptides could significantly inhibit already established HCV infections at the cell levels. This design strategy may be useful in enhancing the bioavailability of other antimicrobial and antiviral peptides [104].

Additionally, two venomous peptides derived from *Heterometrus petersii* have recently been found to effectively inhibit the in vitro infection of herpes simplex virus type 1 (HSV-1). Hp1036 and Hp1239 peptides exhibited remarkable virucidal effects, with EC50 values of 0.43 μM and 0.41 μM, respectively. These peptides demonstrated strong inhibitory action across the attachment, entry, and post-entry stages of the HSV-1 life cycle, indicating their capacity to enter host cells and lower viral contagiousness there. This highlights the potential of Hp1036 and Hp1239 as promising candidates for developing antiviral medications. [105]

Conclusion and Future Prospects:

The advancements in proteomics, genomics, and transcriptomics have made it clear that discovering drugs derived from natural products is a highly effective strategy. While only a limited number of biologically relevant toxins have been extracted from animals that produce these toxins, there remains significant potential to explore and

develop additional therapeutics from these species. Scorpion venom, in particular, is an abundant source of bioactive compounds, including peptides, toxins, and enzymes, which exhibit remarkable molecular activities. Regarding antimicrobial peptides, scorpion venoms have been demonstrated to be a valuable reservoir for AMPs, showcasing various mechanisms and structural characteristics. [106,107] In this review, we have looked after the composition of the scorpion venom encompassing various toxins present (their mechanism of action, amino acid sequence, chemistry and classification), different classes of enzymes and their pharmacological actions and classification of AMPs based on the structure and their chemistry. We have even covered the peptide structure-activity relationships, venom's medicinal use for various microbial infections, and extensive use of the venom for cancer treatment. We focused on various scorpion-derived venom bioactive currently under pre-clinical testing. One of the main challenges in using scorpion venom for clinical purposes is its toxicity and lack of selectivity towards healthy cells. However, advancements in modern technologies now allow for the engineering of toxicity profiles and cell selectivity through specific modifications to the original venom sequences. Several technical challenges must be resolved before venom-based treatments can hit the market. These include acquiring the venom and toxins, characterising the isolates, developing manufacturing methods, and minimising the risk of adverse reactions, especially for long-term use. [108] Despite significant strides in scorpion venomics, our knowledge remains a considerable gap. Further research into scorpion venom's structural and functional aspects is crucial to unlock its complexities and broaden our understanding of possible bioactive substances with medicinal use. [109]

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Author contribution: KS wrote and edited the sections on scorpion venom's chemistry, pharmacology, and structure-activity relationships and its derived peptides. Additionally, he handled the final editing, reference management, and manuscript alignment. SS focused on the recent advancements in the therapeutic potential of scorpion venom in anti-cancer and antimicrobial research, as well as on bioactives currently in pre-clinical trials and citation assistance. KS and SS contributed equally to the manuscript. AL reviewed, edited and corrected the paper. All authors reviewed and approved the final version of the manuscript.

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