



The chemistry of snake venom and its medicinal potential

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Abstract | The fascination and fear of snakes dates back to time immemorial, with the first scientific treatise on snakebite envenoming, the Brooklyn Medical Papyrus, dating from ancient Egypt. Owing to their lethality, snakes have often been associated with images of perfidy, treachery and death. However, snakes did not always have such negative connotations. The curative capacity of venom has been known since antiquity, also making the snake a symbol of pharmacy and medicine. Today, there is renewed interest in pursuing snake-venom-based therapies. This Review focuses on the chemistry of snake venom and the potential for venom to be exploited for medicinal purposes in the development of drugs. The mixture of toxins that constitute snake venom is examined, focusing on the molecular structure, chemical reactivity and target recognition of the most bioactive toxins, from which bioactive drugs might be developed. The design and working mechanisms of snake-venom-derived drugs are illustrated, and the strategies by which toxins are transformed into therapeutics are analysed. Finally, the challenges in realizing the immense curative potential of snake venom are discussed, and chemical strategies by which a plethora of new drugs could be derived from snake venom are proposed.

Neurotoxicity

The ability of a substance to negatively affect the structure or function of the central or peripheral nervous system.

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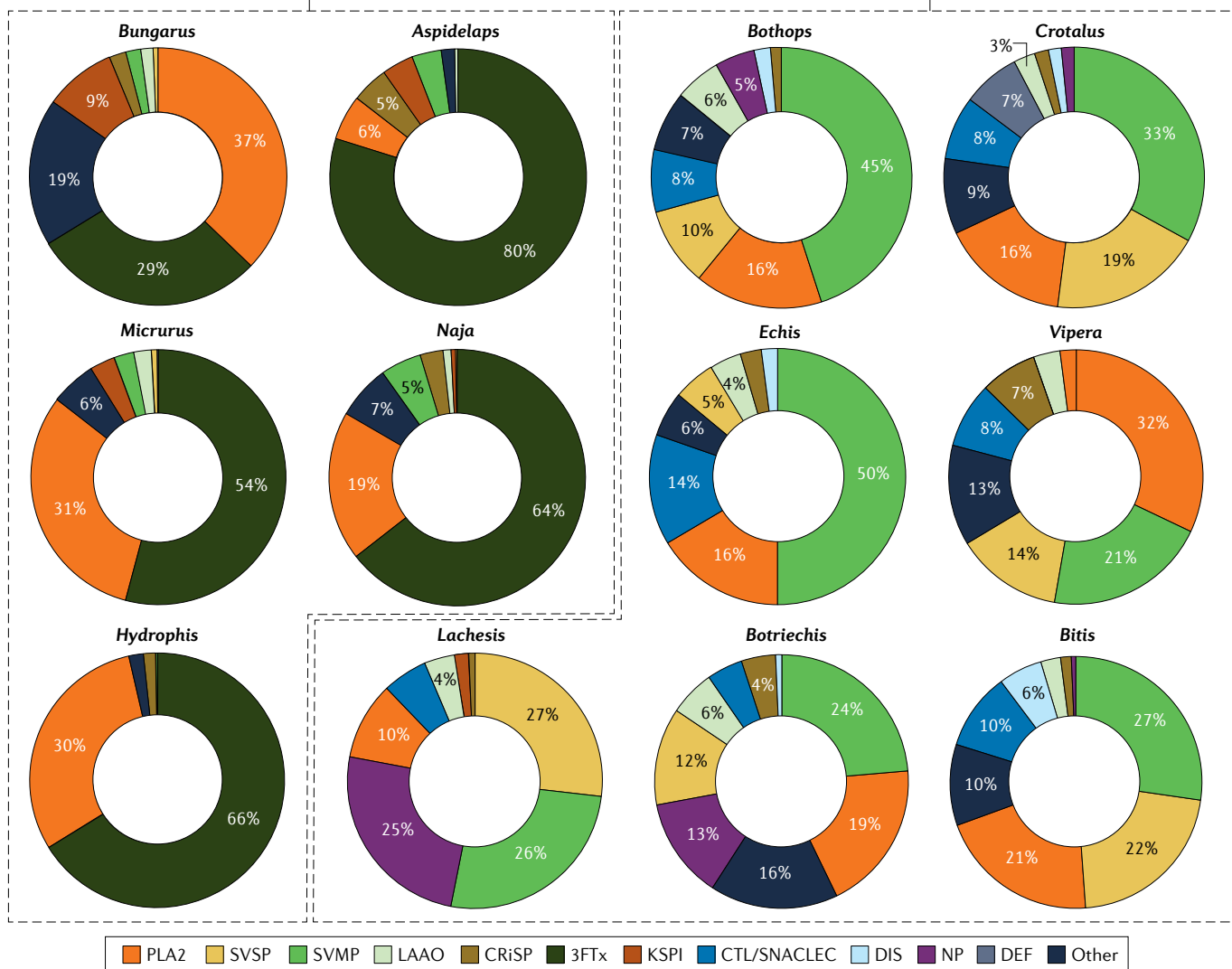
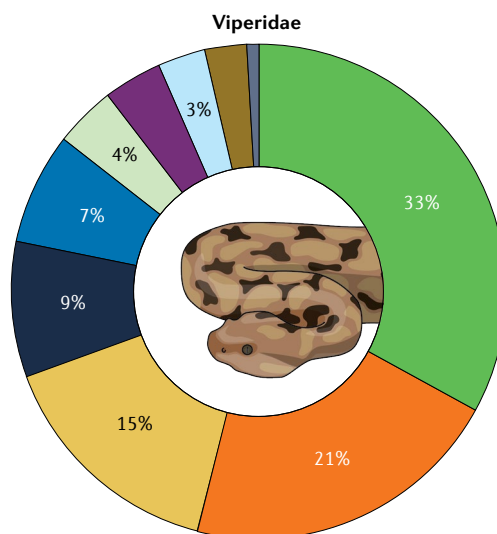
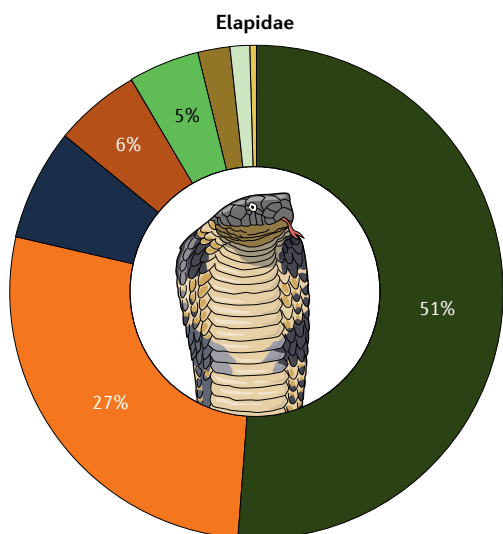
More than 220,000 species, or approximately 15% of all animal diversity on earth, are venomous¹. Venom endows predators with a chemical weapon far more potent than physical force. Animal venoms are complex and sophisticated bioactive cocktails, the main components of which are proteins and peptides². The best characterized animal venoms are probably those derived from cone snails, spiders, scorpions and snakes. The composition of the venoms of the first three is dominated by short (3–9-kDa) disulfide-rich peptides that contain the inhibitor cysteine knot (ICK) motif, although heavier proteins, including enzymes, are also present. ICK peptides are structurally very stable and mainly target the nervous system, acting primarily on membrane channels or neuronal receptors^{3–5}. The venom of a spider or cone snail might contain thousands of different peptides, whereas a scorpion's venom might contain several hundred^{3,6}. The large number of spider species (possibly >100,000) further increases venom diversity.

Snake venoms typically consist of a mixture of 20 to >100 components, of which the majority (>90%) are peptides and proteins⁷, with the dominant bioactivities including neurotoxicity, haemotoxicity and cytotoxicity, depending on the snake species. Venom composition varies widely between species and even within the same

species^{8–15}. Other factors, such as environmental conditions, age, sex or type of prey available, can also affect venom composition¹⁰.

This diversity is a double-edged sword. The only efficient treatment for a snakebite is the administration of the specific antivenom, but the variability in venom composition limits the availability and the upscaling of the production of antivenoms^{16–18}. There are an estimated 2.7 million envenomings each year, which result in >100,000 deaths and leave >400,000 victims with severe and permanent sequelae^{16–19}. However, the compositional diversity is a rich playground for medicinal chemists, providing a collection of highly specific and bioactive compounds that offer many paths towards developing new therapeutic drugs^{1,4,20–22}.

In this Review, we first examine the chemical composition of snake venom, analysing the venoms of >200 snake species. We then discuss the mechanistic details of the chemistry of the principal enzymatic venom toxins. Next, we review the development, structure and mode of action of approved snake-venom-based drugs as well as those of compounds in clinical and pre-clinical testing. Finally, we conclude with an analysis of the vast therapeutic potential of snake venom, pointing out chemical strategies for the transformation of venom into a repertoire of new drugs.



Haemotoxicity
The ability of a substance to negatively affect the cardiovascular system or disrupt haemostasis.

The chemical composition of snake venom

From the middle of the twentieth century, researchers observed the richness in the constituents of venom and began to isolate and analyse the structures and activities of its toxins, as many of them have the potential

to be turned into medicines. Most snake venom toxins belong to one of ~30 families^{23,24}, although the venom of a given snake species can contain hundreds of bio-active compounds^{15,25}. In the snake venoms of known composition, some protein families have many hundreds

◀ **Fig. 1 | Composition of the venom of snakes from the Elapidae and Viperidae families.** The large charts show the averaged composition of the venom of snake species from the Elapidae (elapids) or Viperidae (viperids) families. Each entry in the charts corresponds to a protein family, in which we group tens to hundreds of isoforms. Only protein families with an average abundance of >1% of the total venom proteome are represented, except for the SVSPs in elapids, which are included for comparison with the viperids, and defensins, which although seldom present, can be abundant in the venom of certain species. The distribution of the proportion of the most abundant protein families is shown in Supplementary Fig. 1. Data are from the proteomic studies of the past 15 years; 143 entries for 2007–2017 are from Isbister and Tasoulis's database of snake venom proteomes²; we assembled the additional entries for 2017–2021 from the literature. The Atractaspididae and Colubridae snake families are not included in the study because most are non-venomous or their venoms are weak, not medically important and poorly studied (for venomics studies on colubrids see REF.¹⁵). Each species contributes with the same weight to the average; subspecies or species from different locations were averaged within the entry for the species. The entry 'Other' corresponds to unidentified components or components with an average abundance of <1%. The smaller charts decompose the snake venom composition at the genus level, which reveals the compositional diversity. Only the most well-studied genera are included, which comprise almost all the medically relevant snakes. Supplementary Table 1 details the composition of the venom of each species included in the study together with the relevant reference. 3FTx, three-finger toxin; CRiSP, cysteine-rich secretory protein; CTL/SNACLEC, C-type lectin and C-type lectin-like protein; DEF, defensin; DIS, disintegrin; KSPI, Kunitz-type serine protease inhibitor; LAAO, L-amino acid oxidase; NP, natriuretic peptide; PLA2, phospholipase A2; SVMP, snake venom metalloproteinase; SVSP, snake venom serine protease.

Cytotoxicity

The ability of a substance to negatively affect the structure or function of cells.

Toxins

Toxic compounds produced by a living organism or a virus.

Elapidae

Family of >300 venomous snakes with fixed front fangs. Their venom is often neurotoxic. This family includes the mambas, cobras, coral snakes and most Australian snakes, among others.

Viperidae

Family of >300 venomous heavy-bodied snakes with long, retractable front fangs. Their venom is frequently haemotoxic and cytotoxic. This family includes Old World vipers, rattlesnakes and lanceheads, among others.

Medically important snakes

Snake species that cause notable morbidity and mortality. This classification depends on the venom toxicity, the frequency of snake–human interactions, the aggressiveness of the snake and the health-care facilities.

Myotoxicity

Cytotoxicity specifically directed to myocytes (muscle cells).

of isoforms²⁶. For example, the UniProt database²⁷ contains almost 3,000 isoforms of snake toxins within the reviewed entries. Although there is usually extensive functional redundancy among isoforms of the same protein, there are cases in which different isoforms have different biological activities, which makes their characterization relevant and essential²⁸.

Advances in transcriptomics and proteomics, enabled by advances in mass spectrometry, reverse-phase high-performance liquid chromatography and next-generation sequencing^{15,26,29–31}, have enabled scientists to determine the composition of the venom of hundreds of snake species, giving rise to a field named 'venomics'²⁵ and unveiling the chemical richness of venomous snakes (see the [Reptile Database](#)).

We have reviewed the venomics studies conducted in the past 15 years (2007–2021) for the two most relevant families of venomous snake: Elapidae (elapids) and Viperidae (viperids, commonly referred to as vipers, and further divided into the true viper and pit viper subfamilies). Elapids and vipers include almost all medically important snakes, although there are also some examples in the Colubridae (colubrids) family. FIGURE 1 depicts the averaged venom composition of 76 species and subspecies of the ~400 known elapids and 117 of the ~400 known vipers. Only protein families with an average abundance of >1% of the total venom proteome were considered here. However, because evolutionary and ecological factors can lead to considerable interpopulation and intrapopulation variation in the chemical composition of venom, with numerous exceptions and dichotomies adding to the molecular richness at all taxonomic levels, we also analysed the venom composition of individual snake genera (only the most well-studied were considered), which illustrates the venom diversity. Supplementary Table 1 further illustrates the chemical richness of snake venom by providing the detailed

venom composition for each species and the respective bibliographic sources.

Some toxins act synergistically, and the combination and proportion of each toxin determine the pathophysiology of snakebite envenomation^{17,28}. The difference in the chemical composition of venom from elapids and vipers (FIG. 1) leads to different clinical manifestations. Envenoming by elapids mostly induces neurotoxic, cytotoxic and cardiotoxic manifestations, whereas envenoming by vipers typically induces myotoxicity and haemotoxicity¹⁷.

Elapid venoms mainly comprise peptides and proteins from seven families; secreted phospholipases A2 (PLA2s)^{32–36} and three-finger toxins (3FTxs)^{36–39} are often major constituents and have a dominant role in the action of the venom, although there are many exceptions and considerable diversity at the species level (Supplementary Table 1). For example, the venom of snakes from the *Dendroaspis* genus (mambas) and many Australian snakes show notable exceptions in the composition and action of these toxins. The former lack PLA2 and the latter have a very low content (<6%) of 3FTx. Interestingly, all these snakes have highly potent venoms.

Other toxins — namely snake venom metalloproteinases (SVMPs)^{34,36,40–42}, snake venom serine proteases (SVSPs)^{34,36,43} and L-amino acid oxidases (LAAOs)^{34,44–47} — represent an average of 6% of elapid venom. Kunitz-type peptides⁴⁸ are a family of serine protease inhibitors with the Kunitz domain fold; these peptides constitute an average of ~5% of elapid venom and are potent and selective K⁺-channel blockers. Kunitz-type peptides are particularly prevalent in mambas. The remaining protein families that have been identified appear in smaller amounts.

Viperid venoms mostly include toxins from nine protein families. Again, there are many exceptions and substantial diversity at the individual species and subspecies level (Supplementary Table 1). In most species, the PLA2 (REFS^{32–36}), SVMP^{34,36,40–42,49} and SVSP^{34,36,43,50,51} toxins are dominant, representing an average of ~70% of the whole venom proteome. Most viperid PLA2s are myotoxic, despite sharing extensive sequence identity with the PLA2s of elapids, many of which are neurotoxic. Other toxins that are present in smaller proportions (4–7%) are LAAOs^{34,44–47}, C-type lectins and C-type lectin-like proteins^{52–54}, and natriuretic peptides^{55–57}.

This analysis shows only part of the complexity of snake venom, as hundreds of additional proteins, enzymes and peptides can be present in the venom of each species.

Interspecies variation

Snake venom shows both considerable intraspecies (BOX 1) and interspecies variation. The fraction of PLA2s and 3FTx in the venom of each elapid species varies widely (Supplementary Fig. 1), with the percentage of each ranging from almost 0% to nearly 100%. Interestingly, in most species, a lower fraction of PLA2 is compensated by a higher fraction of 3FTx, and vice versa. Thus, together, they represent, on average, >80% of the total venom proteome in most elapid species. Kunitz-type peptides generally represent <10%, with a more even distribution across species.

Box 1 | Intraspecies variation of snake venom

Venom composition changes within a species owing to age, gender, prey availability, diet and geographic location, among other factors. The venom of the Russell's viper (*Daboia russelii*) is a well known example of this. Its venom has been widely studied owing to the medical importance of this snake. The combination of the high toxicity of its venom, its discreet but aggressive habits and, most importantly, its prevalence in densely inhabited areas mean that the Russell's viper is one of the two snake species that cause more fatalities than any other in the Indian subcontinent, with the other being the saw-scaled viper (*Echis carinatus*), for the same reasons. Together, they make a considerable contribution to the global snakebite burden.

By comparing the abundance of the three major enzymatic toxins in the venom of Russell's vipers from different locations (Supplementary Table 1), it can be seen that the abundance of secreted phospholipases A2 (PLA2s) varies by threefold, that of snake venom serine proteases by threefold and that of snake venom metalloproteinases (SVMPs) by 19-fold.

Another species that shows notable variation in its venom is the common lancehead viper (*Bothrops atrox*), which is the leading cause of snakebite accidents in the Amazon. The venom of common lancehead vipers from Venezuela is composed chiefly of SVMPs (85%), whereas in individuals from Amazonian Peru, Colombia and Pará (Brazil), it constitutes approximately half of the venom only. In individuals in the latter regions, the lack of SVMPs is compensated by an increased abundance of PLA2s^{233–236}. Even among siblings, there is sex-based variation in the venom²³⁷.

An exception is the black mamba (*Dendroaspis polylepis*) — the most feared snake of the African continent. It is very aggressive when threatened, extremely fast, intelligent and has highly toxic and fast-acting venom. Despite this, few fatalities are attributed to this snake, mainly because its habitat is generally far from densely inhabited areas. Black mamba venom primarily consists of Kunitz-type peptides and 3FTxs (61% and 31%, respectively) and lacks PLA2s⁵⁸. Thus, its venom composition is highly atypical, similar to almost no other snake's venom, except for that of the closely related eastern green mamba (*Dendroaspis angusticeps*), which is also devoid of PLA2s and rich in Kunitz-type peptides and 3FTxs, but with the opposite proportions (16% and 69%, respectively). These two mambas may represent the most outstanding examples of the chemical diversity of elapid venoms.

The composition of viperid venom varies widely across genera (FIG. 1) and species (Supplementary Fig. 1 and Supplementary Table 1). The PLA2, SVSP and SVMP enzymatic families represent an average of ~70% of the viperid proteome. The proportion of PLA2 ranges from almost zero to >90%, with the distribution peaking at ~10% (Supplementary Fig. 1). The SVMP ratio also has a broad distribution, with a peak at ~40%. SVSPs generally constitute <20%, although there are species, such as the Okinawa pit viper (*Ovophis okinavensis*), whose venom consists almost entirely of SVSPs⁵⁹. Although present in low quantities on average, C-type lectins and C-type lectin-like proteins, defensins or natriuretic peptides can represent >37% of the viperid proteome in specific species.

Defensins are small proteins ubiquitous across life that function as host defence peptides and have antimicrobial and/or immune signalling activities. The defensins found in viperid venom act on the Na⁺ and K⁺ channels of plasma membranes, including that of muscle cells (the sarcolemma), and accumulate in the lysosomes, causing analgesic, neurotoxic, myotoxic and cytotoxic effects^{60,61}. The accumulation in the lysosomes

is an unusual mechanism of cytotoxicity among snake toxins. Furthermore, the structures of defensins are unlike any other channel-binding toxins. Although they can be more abundant in specific venoms, defensins are typically rare, and constitute on average 1% of venom.

In conclusion, our analysis confirms and further reinforces the understanding of the incredible diversity of snake venom. This diversity of highly bioactive proteins and peptides, which recognize essential biological targets with exquisite specificity and affinity, constitutes a unique pharmacological database for drug discovery.

Chemistry of the major enzymatic toxins

Since the first identification and characterization of the structure of a snake venom toxin, crotoxin from *Crotalus durissus terrificus* in 1938 (REF.⁶²), there has been intense research aimed at elucidating the structure, reactivity and target of venom toxins, in order to understand their function and determine their molecular determinants of recognition and therapeutic potential. TABLE 1 summarizes the information currently available for several prominent toxin families and provides suggestions for further reading. In this section, we discuss the reaction chemistry of the three principal enzymatic toxin families: PLA2s, SVMPs and SVSPs. Some of the other abundant toxins without catalytic activity are discussed in the next section.

Secreted phospholipases A2

PLA2s exist as active enzymes and as inactive PLA2-like proteins in snake venom. PLA2 enzymes catalyse the hydrolysis of the sn-2 ester bond of cell-membrane phospholipids and are classified into 14 groups, from which the groups IA and IIA are present in elapid and viperid venoms^{33,35}. The enzymes in these groups have a molecular mass of 13–19 kDa, contain 5–8 disulfide bridges and form dimers in aqueous solution⁶³. In cell membranes, PLA2s dissociate and bind as monomers^{63,64}, and show an affinity for membrane regions in which at least 15% of the phospholipids are negatively charged⁶⁵ (FIG. 2a). PLA2s can also be divided into acidic and basic isoforms, according to the isoelectric point (pI), with the basic isoforms having a higher membrane affinity and thus higher toxicity³².

The catalytic mechanism of PLA2s is still unclear at the atomic level. Nevertheless, it is a two-stage process, with the first corresponding to the binding of the PLA2 to the membrane and the second to the chemical reaction. The first stage determines the enzyme–target specificity, whereas both stages determine the enzyme efficiency⁶³. The catalytic activity of PLA2s depends on Ca²⁺, which stabilizes the tetrahedral transition state of the reaction^{65,66}. An essential aspartate residue (Asp49) coordinates the Ca²⁺ cofactor, the mutation of which renders the enzyme inactive. As metadata analyses have shown, it is unusual for Ca²⁺ cofactors to participate in the catalytic cycle^{67,68}. Other divalent metal ions that are larger or smaller, or harder or softer, than Ca²⁺ lead to a notable drop in activity⁶⁹ for a reason not yet understood. In the most-accepted mechanism⁶⁵, the active site histidine residue (His48) abstracts a proton from a water molecule bound to the Ca²⁺ ion, and the resulting hydroxy group

C-type lectins

Superfamily of >1,000 proteins, most of which bind carbohydrates in a Ca²⁺-dependent manner. The proteins share a C-type lectin-like domain in their carbohydrate-binding region. In snake venoms, they are haemotoxic.

C-type lectin-like proteins

A protein family whose members feature a domain with the C-type lectin fold, which lacks critical structural elements to recognize and bind sugars. In snake venoms, these proteins are haemotoxic.

Sarcolemma

Specialized type of cell plasma membrane that surrounds muscle cells. It is frequently the target of snake venom myotoxins.

Lysosomes

Membrane-bound organelle containing digestive, hydrolytic enzymes whose function is primarily the degradation of macromolecules, old cell parts and microorganisms. Lysosomes represent the waste disposal of a cell.

Table 1 | Characteristics of the main families of snake venom toxins

Toxin family	Snake family	Enzymatic activity	Principal biological targets	Major pathophysiological activities	Most promising therapeutic applications	Representative toxin structures	Further reading
PLA2	Elapids and viperids	Yes	Plasma membrane of myocytes and various receptors in the axolemma (undetermined molecular target)	Acute skeletal muscle necrosis, flaccid paralysis, local inflammatory reactions (oedema, leukocyte influx into tissues and pain)	Antibacterial activity against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> and <i>Enterobacter aerogenes</i> ¹⁹² ; anti-parasite effects ¹⁵⁵ ; antiviral activity against HIV ^{193,194} and dengue ¹⁹⁵	5TFV (basic); 1JIA (basic); 1Y4L (PLA2 homologue)	32–36
SVMP	Elapids (P-III SVMPs) and viperids (P-I, P-II and P-III SVMPs)	Yes	Wide variety of targets; most notable are collagen IV and blood coagulation factors	Predominantly, haemorrhagic activity but can cause the proteolytic degradation of fibrinogen and fibrin, induce apoptosis and inhibit platelet aggregation	Haemostasis; blood coagulation, fibrinolysis and platelet aggregation ^{78,168,196}	2W15 (P-I bound to a peptidomimetic); 2M75 (P-II, DIS domain); 3DSL (P-III)	34,36,40–42,49
SVSP	Elapids and viperids	Yes	Mostly blood coagulation factors	Imbalances the haemostatic system through action in the coagulation cascade on the fibrinolytic and kallikrein–kinin systems	Prevention of thrombus formation through fibrinogen depletion; anticoagulant ^{197,198} ; applied in neurosurgical ¹⁹⁹ and vertebro-spinal ²⁰⁰ procedures as a fibrin sealant	1OP0 (glycosylated); 3S9B (RVV-V in open form); 3S9C (RVV-V bound to factor V)	34,36,43,50,51
3FTx	Elapids	No	Nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase and cardiomyocytes (undetermined molecular target)	Neurotoxic effects, which cause paralysis; muscle fasciculations; and cardiac arrest through lysis of cardiomyocytes	Regulation of blood pressure ²⁰¹ ; treatment of coagulation disorders ^{202–204} ; analgesic action ^{146,151,154}	1QKD (short-chain α -neurotoxin); 1Y15 (long-chain α -neurotoxin); 1KBA (κ -neurotoxin dimer); 3PLC (β -cardiotoxin); 1F8U (receptor-bound fasciculin); 4DO8 (muscarinic toxin)	36–39
LAAO	Elapids and viperids	Yes	L-amino acids; substrate varies among species	Haemorrhagic or anticoagulant effects, induction of apoptosis, oedema and platelet aggregation or inhibition	Antimicrobial against <i>P. aeruginosa</i> , <i>Candida albicans</i> , <i>S. aureus</i> ; antiparasitic against <i>Leishmania chagasi</i> and <i>Leishmania amazonensis</i> ^{205,206} ; potential antiviral against HIV-1 (REF. ²⁰⁷)	2IID (with L-phenylalanine and its cofactor FAD, and glycosylated); 4E0V (with cofactor FAD); 5T55 (glycosylated, and with cofactor FAD)	34,44–47
CRISP	Elapids and viperids	No	Ca ²⁺ channels, K ⁺ channels, and signalling cascades involved in cell adhesion	Inhibits angiogenesis, increases vascular permeability and promotes inflammatory responses (leukocyte and neutrophil infiltration)	Antiparasitic against <i>Leishmania</i> and trypanosome strains ²⁰⁸ ; antimicrobial against Gram-negative bacteria and filamentous fungus ²⁰⁹	6IMF (bound to inhibitor peptide); 1WVR (Ca ²⁺ -channel blocker triflin); 3MZ8 (zinc-bound natrin)	210
CTL/ SNACLEC	Viperids	No	Platelet and cellular receptors, as well as coagulation factors, such as factor IX and factor X	Diverse effects, including haemagglutination, mitogenic activity, platelet aggregation, oedema, elevated vascular permeability, renal effects, hypotension, cytotoxicity and modulation of Ca ²⁺ release from skeletal muscle sarcoplasmic reticulum	Use in anticoagulant therapies ^{211–213}	1JZN (CTL in complex with galactose); 1UOS (CTL convulxin); 3UBU (SNALEC bound to platelet glycoprotein Ib)	52–54

Table 1 (cont.) | Characteristics of the main families of snake venom toxins

Toxin family	Snake family	Enzymatic activity	Principal biological targets	Major pathophysiological activities	Most promising therapeutic applications	Representative toxin structures	Further reading
DIS	Viperids	No	Integrins	Disrupts cell–cell adhesion and cell–matrix adhesion, and inhibits angiogenesis	Anti-inflammatory and antiangiogenic for chronic inflammatory processes ²¹⁴ ; template in the development of the anti-platelet marketed drugs eptifibatid and tirofiban to treat thrombosis ²¹⁵ ; treatment for neoplastic processes ²¹⁶	1J2L (trimestatin); 3C05 (acostatin); 1RMR (schistatin)	107,217–219
NPs	Elapids and viperids	No	NP receptors A, B and C	Potent hypotensive effects (vascular relaxation and a decrease in myocardial contractility), leading to rapid loss of consciousness	Cardiorenal diseases; heart failure ¹⁴⁴	4AA2 (BPPb bound to an ACE-I homologue); 4APJ (BPPb bound to ACE-I)	55–57
KSPI	Elapids and viperids	No	Proteases and K ⁺ channels	Inhibition effects on a range of serine proteases, including plasmin and trypsin, leading to anticoagulation, fibrinolysis, inflammation and ion-channel blocking	Reduction of cyst development in polycystic kidney diseases through inhibition of vasopressin type 2 receptor pathways ²²⁰	3BYB (textilinin-1); 3D65 (textilinin-1 in complex with trypsin); 5M4V (mambaquaretin-1)	48
DEF	Elapids and viperids	No	Skeletal muscle Na ⁺ and K ⁺ channels, lipid membranes and sarcolemma	Myotoxic damage through depolarization of skeletal muscles, and analgesic activity	Anti-proliferative, anti-nociceptive, anti-inflammatory, antifungal against <i>C. albicans</i> , anti-Plasmodium, anti-Leishmanial and anthelmintic ^{129,221–224}	4GV5 (crotamine, X-ray); 1H5O (crotamine, NMR)	60,61

Only toxins from the elapid and viperid families are included. Within each family, there are tens or hundreds of different isoforms. Although there are numerous species-specific and isoform-specific exceptions, the most common biological target(s) and pathophysiological manifestations are reported. Representative structures, when available, are taken from the Protein Data Bank. Additional details on each toxin can be found in the referenced articles. We also refer readers to REF.²²⁵, which is an excellent and detailed source of information on the structure, bioactivity, pathophysiology and therapeutic applications of snake venom toxins. 3FTx, three-finger toxin; ACE-I, angiotensin-1 converting enzyme; BPPb, bradykinin potentiating peptide b; CRiSP, cysteine-rich secretory protein; CTL/SNACLEC, C-type lectin and C-type lectin-like; DEF, defensin; DIS, disintegrin; KSPI, Kunitz-type serine protease inhibitor; LAO, L-amino acid oxidase; NMR, nuclear magnetic resonance; NP, natriuretic peptide; PLA2, phospholipase A2; RVV-V, factor-V activating enzyme from Russell's viper venom; SVSP, snake venom serine protease; SVMP, snake venom metalloproteinase.

attacks the sn-2 ester bond. A chain of water molecules probably mediates the proton transfer to His48.

In a twist of evolution, viper PLA2s split between enzymes and catalytically inactive proteins, known as PLA2 homologues. The latter lack the Ca²⁺ cofactor owing to substitution of the Asp49 residue by lysine or, less commonly, by serine, arginine, glutamine or asparagine. The (Lys49) PLA2 homologues are highly myotoxic, despite having no enzymatic activity. A sequence of ~12 positively charged and hydrophobic residues at the C-terminal region, with positive ends and a mixed positive and hydrophobic core (such as the KKRYRYLKLPLCKK sequence in MT-II from the venom of terciopelo (*Bothrops asper*)), is believed to penetrate and destabilize the sarcolemma (FIG. 2b), and thus promote an influx of Ca²⁺ ions, which starts a chain of harmful events that leads to myotoxicity^{32,70,71}. Further investigation is needed to achieve an atomic-level understanding of this effect. This stretch of residues alone often has similar bioactivity to that of the whole protein and is intensively investigated as a model to construct antimicrobial peptides^{69,72–74}.

Some neurotoxic PLA2s have even more refined molecular mechanisms of action that serve as lessons for drug delivery. An example is β -bungarotoxin from the venom of the Taiwan banded krait (*Bungarus multicinctus*), which comprises a PLA2–Kunitz-type peptide heterodimer. The toxin travels silently through the victim's body, avoiding off-target membranes owing to the partially occluded PLA2 active site and its low membrane affinity. When β -bungarotoxin reaches the pre-synaptic region of the neuromuscular junction, the Kunitz-type peptide recognizes and binds a specific K⁺ channel, trapping the toxin at this location. This event exerts a first neurotoxic action. The PLA2 monomer, once firmly anchored at the membrane, opens the active site and initiates hydrolysis of the membrane phospholipids, near the K⁺ channel, further enhancing the neurotoxic effect^{75–77} (FIG. 2c).

In summary, PLA2 enzymes and their inactive homologues share extensive sequence and structural similarity, and both induce myotoxicity but through surprisingly diverse molecular mechanisms. The great diversity of PLA2 isoforms translates into a surprising

Neuromuscular junction

A specialized synapse established between a motor neuron and a muscle fibre through which signals for muscle contraction are transmitted.

Fibrinogen

A protein complex in the plasma of vertebrates that is enzymatically and sequentially converted into fibrin and a fibrin-based blood clot. Fibrinogen is responsible for stopping bleeding from blood vessels.

variety of biological activities, underlying molecular machinery and recognition targets, and, consequently, a variety of drug discovery opportunities⁷³.

Snake venom metalloproteinases

SVMPs are mostly haemorrhagic and are classified into three groups (P-I to P-III) according to the number of domains (1–3), with further division into subgroups^{40,42}. P-III SVMPs are the largest, more ancient and most complex enzymes, from which the P-II and P-I enzymes evolved through domain loss. Elapid venoms contain only P-III SVMPs. By contrast, viperid venoms contain SVMPs from each of the three groups, with SVMPs being a prominent toxin, and often the most abundant one (FIG. 1).

P-I SVMPs comprise the catalytic domain only, which catalyses the hydrolysis of a vast array of physiologically relevant enzymes and structural proteins. This domain is common to the three SVMP groups. Its hydrolytic targets include collagen IV, fibrinogen and coagulation factors, with extensive haemorrhagic consequences^{41,49}. Hydrolysis of collagen IV weakens capillary walls, which causes them to collapse under otherwise normal

haemodynamic pressure⁴¹. Continuous hydrolysis of fibrinogen in vivo leads to weak, inefficient fibrin clots and hypofibrinogenaemia, and the hydrolysis of blood coagulation factors deregulates blood clotting^{78,79}.

P-II SVMPs have an additional disintegrin domain that inhibits platelet aggregation through specific binding to the blood platelet $\alpha_{IIb}\beta_3$ integrin — a vital protein that triggers fibrinogen binding and platelet aggregation⁸⁰. This action reinforces the haemorrhagic effect of collagen IV hydrolysis.

P-III SVMPs (FIG. 3) have a catalytic domain, a disintegrin-like domain with a collagen-binding three-amino acid Glu–Cys–Asp (ECD) motif (instead of the typical P-II Arg–Gly–Asp (RGD) motif) and a cysteine-rich domain. The prominent role of the latter is substrate recognition and binding. Nevertheless, the catalytic domain is also involved in substrate recognition through an interesting conformational selection mechanism (BOX 2). In some isoforms, a C-type lectin-like domain is also present⁴⁰.

The reaction mechanism of SVMPs is not fully clarified despite a wealth of X-ray structures^{81–83}.

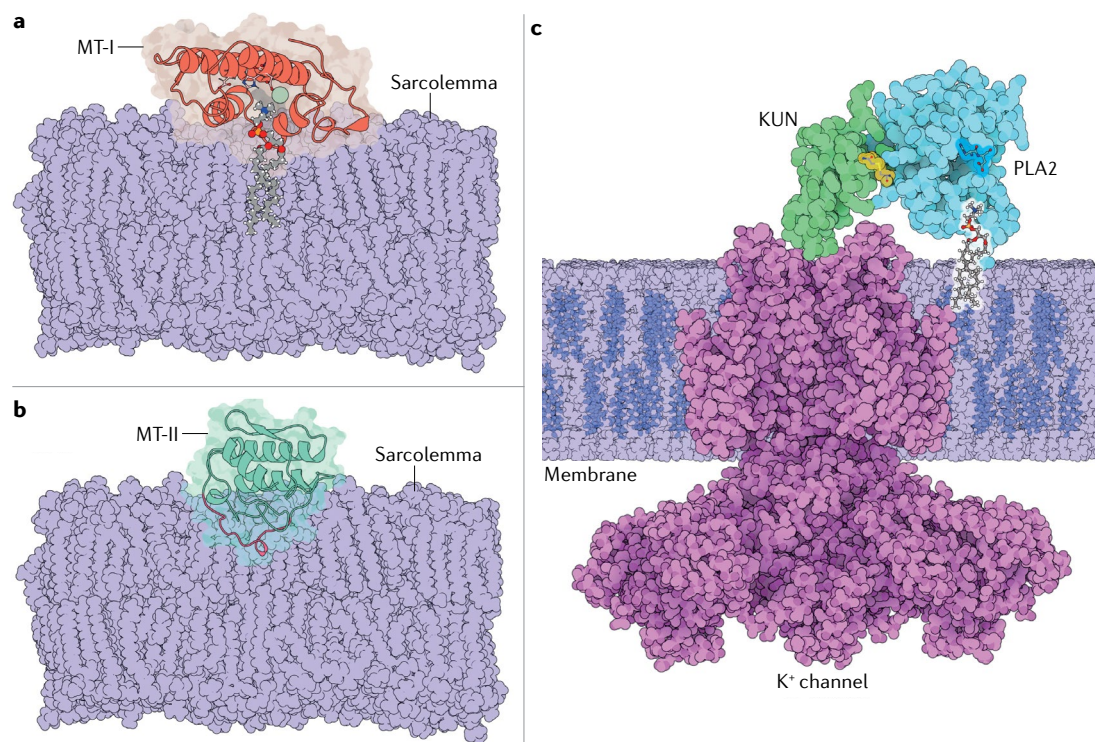


Fig. 2 | The three main types of PLA2 bound to their targets. a | Myotoxin I (MT-I), a strongly myotoxic phospholipase A2 (PLA2) from the venom of a terciopelo viper (*Bothrops asper*), attached to the sarcolemma. MT-I (PDB ID: 5TFV)²²⁶ is shown with a phospholipid substrate bound to the active centre. In the phospholipid, oxygen is red, phosphorus is orange, nitrogen is blue, carbon is grey and hydrogen is white; the enzyme is shown in light red, and the Ca²⁺ ion is shown in light green. The residues that form the protein–membrane interface and the PLA2–membrane binding geometry were identified through mutagenesis, fluorescence and X-ray crystallography studies^{54,65}. **b** | The PLA2 homologue myotoxin II (MT-II), also from terciopelo venom (PDB ID: 1Y4L)²²⁷, bound to the sarcolemma. The C-terminal region destabilizes and permeabilizes the membrane⁷⁰. The protein is shown in light green, and the C-terminal KKYRYLKLPLCKK sequence is shown in pink. **c** | β -Bungarotoxin (PDB ID: 1BUN)⁷⁵ from the Taiwan banded krait (*Bungarus multicinctus*) bound to a neuronal membrane. The toxin travels silently through the victim's body until its Kunitz (KUN) domain (green) recognizes and binds a presynaptic voltage-gated K⁺ channel (violet, PDB ID: 6PBX) with high specificity, trapping the PLA2 domain (light blue) at the neuronal membrane, where its active site, otherwise occluded, opens and starts degrading the adjacent phospholipids (bound phospholipid coloured by element)^{75–77}.

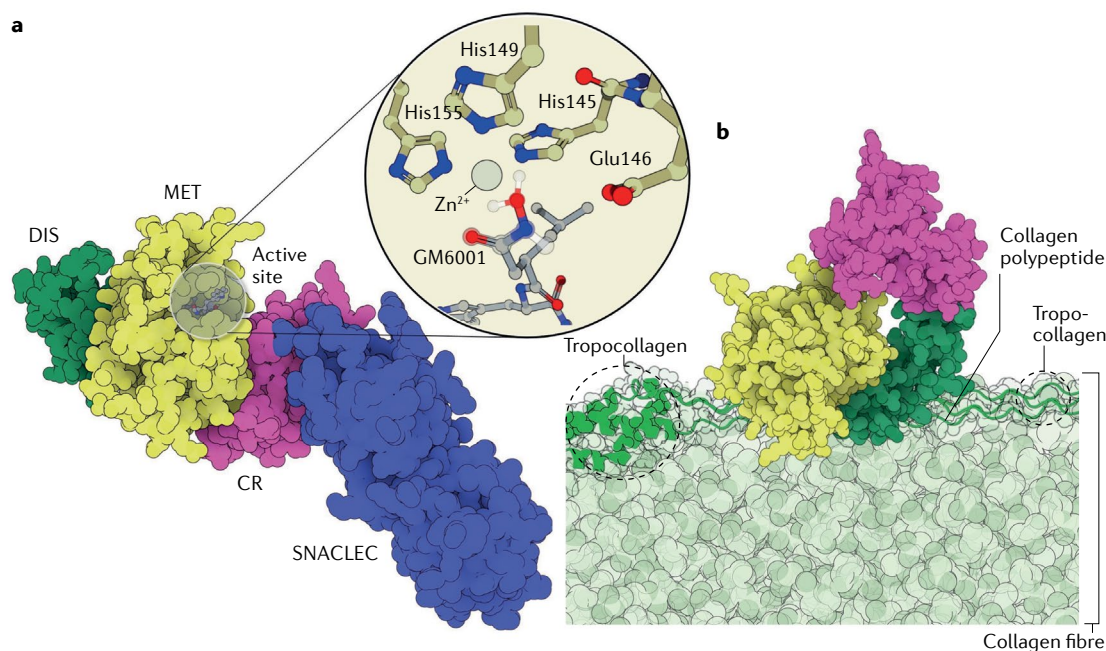


Fig. 3 | Structures of SVMPs and their substrates. a | The structure of the factor X activating enzyme RVV-X (PDB ID: 2E3X)⁸¹ from the eastern Russell's viper (*Daboia siamensis*). RVV-X is a P-III snake venom metalloproteinase (SVMP) isoform that is ubiquitous in species from the Indian subcontinent²²⁸. RVV-X activates blood coagulation factor X by hydrolysing the Arg194–Ile195 position with such high specificity that it is used as a diagnostic tool for haematologic disorders^{26,93,94,229}. The catalytic domain (MET) is coloured yellow, the disintegrin (DIS) domain is coloured green and the cysteine-rich domain (CR) is coloured pink. RVV-X has an additional C-type lectin and C-type lectin-like protein (CLT/SNACLEC) domain, which is shown in blue. The inset shows the Zn²⁺ cofactor with its coordination shell and the peptidomimetic inhibitor GM6001, whose two coordinated oxygen atoms mimic the positions of the water molecule and the carbonyl of the substrate (superimposed on top of GM6001 with a translucent ball and stick representation). **b** | Illustrative scheme of daborhagin²³⁰, a highly haemorrhagic SVMP from Russell's viper venom, bound to collagen IV at the basement membrane of capillaries. The colour scheme of the enzyme domains is the same as that of RVV-X in part **a**. A collagen IV fibre is shown in light green, with a tropocollagen unit emphasized in dark green and drawn in a cartoon and tube representation. The hydrolysis of collagen IV weakens the mechanical stability of the capillary wall, which breaks down under regular haemodynamic forces, leading to massive haemorrhage. Daborhagin was modelled with the active site facing collagen IV.

The mechanism is proposed⁸² to begin with the coordination of the scissile carbonyl of the substrate to the Zn²⁺ ion in the active site, such that the carbonyl group is held at an attacking distance from a water molecule also bound to the Zn²⁺ centre. Following deprotonation of the water molecule by a conserved glutamate residue (Glu146), the resulting hydroxide ion attacks the carbonyl carbon to form a Zn²⁺-bound gem-diolate. Finally, the neutral Glu146 protonates the peptide amine, leading to cleavage of the peptide bond.

Snake venom serine proteases

SVSPs are primarily haemotoxic and interfere with blood coagulation, blood fibrinogen levels, blood pressure and platelet aggregation^{34,36,43,50,51} (FIG. 4a,b), although there is one known example of an SVSP with K⁺-channel blocking activity⁸⁴ (FIG. 4c). The resistance of SVSPs to endogenous serine protease inhibitors endows them with their toxic effects. Many of the activities of SVSPs mimic those of the enzyme thrombin, which is a vital component of the blood coagulation cascade. Each SVSP exhibits one or more of the activities of thrombin and sometimes has bioactivities that thrombin does not. But no SVSP possesses all the bioactivities of thrombin⁸⁵, which makes them toxic and, in contrast to thrombin,

SVSPs deregulate homeostasis^{43,85}. SVSPs that share some of the fibrinolytic activities of thrombin have been named thrombin-like enzymes.

SVSPs are monomeric glycoproteins with ~228–239 residues and a molecular mass of 26–67 kDa (REFS^{34,43}). This wide range of molecular masses is due to different patterns of *N*-glycosylation and *O*-glycosylation. The enzymes share the typical trypsin fold and the highly conserved Ser195–His57–Asp102 catalytic triad (chymotrypsin numbering) (FIG. 4). Six disulfide bonds stabilize the structures.

Most SVSPs share the classical reaction mechanism of serine proteases. However, >20 SVSPs with variations in the canonical catalytic triad have been found in snake venom transcripts⁸⁶. Among the few of these that have been characterized, the horned viper (*Vipera ammodytes ammodytes*) serine protease VaSP1, which bears the rare Ser195–Lys57–Asp102 triad, was surprisingly found to be catalytically active⁸⁷, illustrating an unexpected richness in SVSP chemistry.

In contrast to thrombin, which activates many different coagulation factors (factor V, factor VIII, factor XI and factor XIII, as well as fibrinogen), each SVSP is highly substrate-specific⁵⁰. However, as different SVSPs are specific for different sets of targets, a group

Intrathecal administration

Invasive drug administration by injection through the skull or the spine, allowing the drug to reach the cerebrospinal fluid, and thus the brain, without crossing the blood–brain barrier.

of isoforms can induce diverse physiological manifestations. This recognition diversity is striking given their extensive mutual sequence identity (50–85%), which is a phenomenon known as the identity–selectivity paradox⁵⁰; their specificity cannot be understood from the primary sequence. Instead, the specificity appears to depend on a combination of subtle structural epitopes, primary and secondary binding sites, enzyme flexibility, glycosylation and water organization⁵⁰. The precise specificity and intense haemoactivity of SVSPs make them potential diagnostic and therapeutic tools in the cardiovascular area.

The chemistry of snake venoms is partially understood for the major enzymes, but further understanding at the atomic level is required. Computer simulations are one of the best ways to answer remaining questions, particularly given the recent advances in quantum mechanical and classical mechanics methods, which enable the reliable prediction and determination of complex chemical reaction mechanisms^{88–91}.

Drugs from snake venom toxins

Snake venom finds three major therapeutic applications: pharmaceutical drugs^{4,92}, toxin-based diagnostic methods^{92–94} and biological markers for understanding human physiology²⁶. We focus here on pharmaceutical drugs based on snake venom. This section discusses the snake venom toxins and toxin-inspired molecules that are being used to develop new drugs, focusing on the drugs approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as well as drugs under development in preclinical and clinical trials.

Approved drugs

Snake venoms are typically cytotoxic, neurotoxic and haemotoxic. The anticancer potential of cytotoxins has long been recognized⁹². Neurotoxins are of interest for the treatment of neurological diseases. However, no drug derived from a snake venom neurotoxin has yet reached the market. The complexity of the human neurological system, our insufficient understanding of this system and the difficulty in delivering medications to the nervous system contribute to the slow progress of this line of drug discovery²⁰. Nevertheless, the FDA and EMA approved ziconotide, a ω -conotoxin peptide from the magic cone snail (*Conus magus*), as an analgesic for severe chronic pain^{4,21,95,96}. The main limitation of this drug is its intrathecal administration route. In contrast to neurotoxins, haemotoxins have given rise to numerous

drugs approved by the FDA and EMA, in part because they affect a system whose physiology is well known and easier to manipulate. As cardiovascular diseases are the leading cause of death globally, the development of snake-venom-derived drugs that target the cardiovascular system is appealing.

Captopril. The antihypertensive drug captopril was the first drug based on a bioactive component from snake venom that was approved in the US by the FDA in 1981 and in European countries from 1984 onwards. The realization that envenoming by the South American pit viper jararaca (*Bothrops jararaca*) caused notable hypotension led to the discovery of the vasodilator peptide bradykinin in its venom⁹⁷. Subsequent studies led Sérgio Ferreira and colleagues to discover a set of nine peptides in the venom of jararaca that potentiated the effect of bradykinin, named bradykinin potentiating factors (BPFs)^{98–100}. BPFs inhibit the angiotensin-converting enzyme (ACE)¹⁰¹, which otherwise degrades bradykinin. The therapeutic potential of BPFs led the pharmaceutical company Squibb to develop a drug against hypertension using BPF peptides (BPP_{5a} and BPP_{9a}, in particular) as templates¹⁰² (FIG. 5a). The result was captopril, a small, synthetic, orally bioavailable and potent bioactive molecule with a structure and electrostatics that mimic the BPP_{5a} Pro–Ala–Trp recognition motif for ACE.

Captopril was a milestone in many ways: it was the first drug developed from animal venom; it was created by converting toxic action into therapeutic action; it was one of the first examples and a paradigm of ligand-based drug discovery; and it was the first drug targeting ACE, rapidly becoming a blockbuster and saving countless lives¹⁰⁰.

To overcome the side effects of captopril caused by its thiol group, Merck developed enalapril^{102,103} (FIG. 5a). The thiol group in captopril was replaced by a carboxylate, leading to a loss of potency, which was compensated with additional modifications. The resulting compound (enalaprilat) lacked oral bioavailability, most probably owing to the ionic carboxylate. Enalaprilat was converted into its ethyl ester to overcome the problem, giving rise to enalapril, a prodrug with very good oral bioavailability^{21,104} and approved by the FDA and EMA. Enalapril became Merck's first billion-dollar-selling drug in 1988.

Many ACE-inhibitor drugs based on the BPP_{5a} binding motif were subsequently developed and approved. Examples include lisinopril, quinapril, ramipril,trandolapril and moexipril^{105,106}, which, despite being frequently dismissed in the snake-based drug discovery world, deserve to be considered snake-venom-based drugs. These drugs are among the most prescribed globally and showcase the immense therapeutic potential of venoms, which is yet to be fully realized.

Tirofiban. Tirofiban is an antiplatelet drug approved by the FDA in 1998 and the EMA in 1999 for treating acute coronary syndrome^{107–110}. Its structure is derived from the toxin echistatin¹¹¹, a 49-residue disintegrin from saw-scaled viper (*Echis carinatus*) venom. Echistatin competes with fibrinogen for binding to the $\alpha_{IIb}\beta_3$ integrin,

Box 2 | Substrate recognition by snake venom metalloproteinases

Despite extensive sequence identity, only some snake venom metalloproteinases (SVMPs) bind collagen IV and have haemorrhagic activity. Molecular dynamics simulations explained this paradox by revealing that in haemorrhagic SVMPs, the first half of the Ω -loop (residues 156–165) is highly flexible, whereas in non-haemorrhagic SVMPs, the second part of the Ω -loop (residues 166–175) is flexible instead. On the basis of this observation, the authors proposed that the flexibility of this loop is crucial for collagen IV recognition²³⁸ and, more recently, this hypothesis has been experimentally confirmed²³⁹. Thus, the lesson here is that target recognition might rely on protein dynamics and not only on the static X-ray structure; this constitutes a formidable challenge for drug discovery.

which inhibits the final step in platelet aggregation⁹². Echistatin thus reinforces the haemorrhagic activity of saw-scaled viper SVMPs.

Echistatin shares the RGD motif of the disintegrin domains of many P-II-type SVMPs, which is the

minimal sequence for $\alpha_{\text{IIb}}\beta_3$ recognition. It binds several integrins with sub-nanomolar affinity, with selectivity for $\alpha_{\text{IIb}}\beta_3$ over others¹⁰⁷. In high concentrations, the isolated RGD tripeptide also inhibits platelet aggregation. Tirofiban was modelled to replicate the RGD

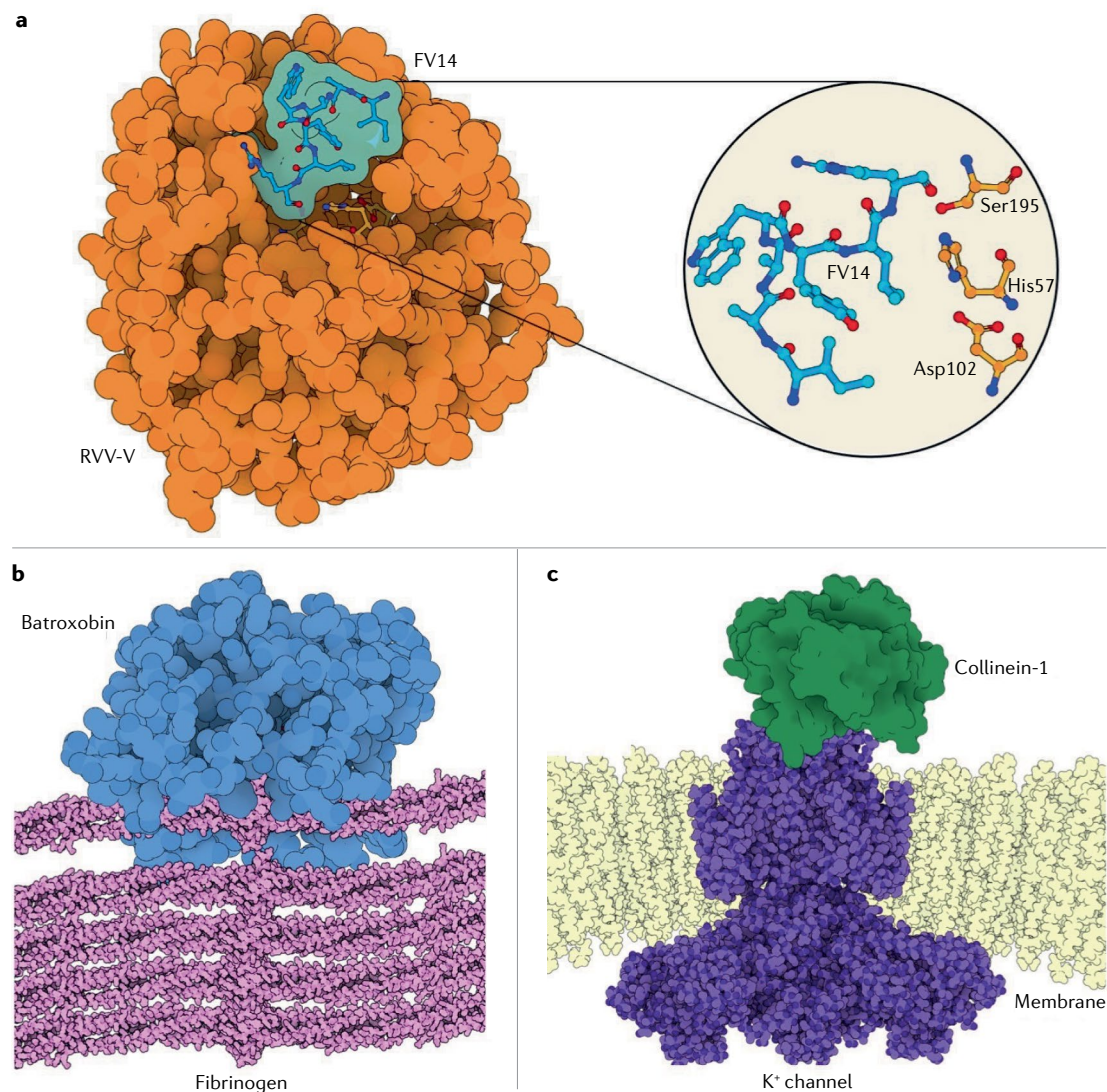


Fig. 4 | Structure of SVSPs and their substrates. a | The factor V activating enzyme from Russell's viper venom (RVV-V; PDB ID: 3S9C), which is a snake venom serine protease (SVSP) with specificity for blood coagulation factor V. RVV-V is depicted in a complex with the 14-residue terminal fragment of factor Va (residues 1533–1546), called FV14. RVV-V releases the last 61 residues of factor V by hydrolysing its Arg1545–Ser1546 bond, generating procoagulant factor Va and mimicking one of the physiological roles of thrombin²³¹. The inset shows the active site and factor V hydrolysis product. RVV-V recognizes factor V through a selective induced-fit mechanism that opens an otherwise closed subpocket. The strict specificity of RVV-V for factor V makes it a useful diagnostic tool for measuring factor V levels, lupus anticoagulant levels and resistance to activated protein C^{93,94}. **b** | Illustrative representation of the thrombin-like Brazilian lancehead pit viper (*Bothrops moojeni*) SVSP batroxobin (Defibrase)^{4,21,118,119,232} bound to fibrinogen. Thrombin cleaves the A α and the B β chains of fibrinogen and converts factor XIII into factor XIIIa, which generates crosslinked fibrin, whereas most SVSPs cleave either the A α or the B β chain only⁸⁵. Batroxobin cleaves only the A α chain^{118,232}. SVSPs therefore form abnormal, easily degradable fibrin clots that lead to fibrinogen depletion and hypofibrinogenaemia. The clotting time in the presence of batroxobin (reptilase time) is used in the clinic to diagnose several diseases^{93,94}. Batroxobin was modelled from the homologue saxthrombin (PDB ID: 3S69). **c** | Collinein-1 from the neotropical rattlesnake (*Crotalus durissus collilineatus*) is the first example of an SVSP with specific K⁺-channel blocking activity⁸⁴. Through a mechanism that is independent of its enzyme activity, collinein-1 selectively inhibits the oncogenic hEAG1 channel (PDB ID: 6PBX) among 12 tested voltage-gated K⁺-channels, with obvious antitumour implications. As K⁺ channels are known targets for many animal neurotoxins, the discovery of collinein-1 makes it tempting to speculate that some yet unknown SVSP isoforms might have found a neurotoxic role. Collinein-1 was modelled from the homologue thrombin-like enzyme AhV_TL-I (PDB ID: 4E7N) and is illustratively bound to the oncogenic hEAG1 channel.

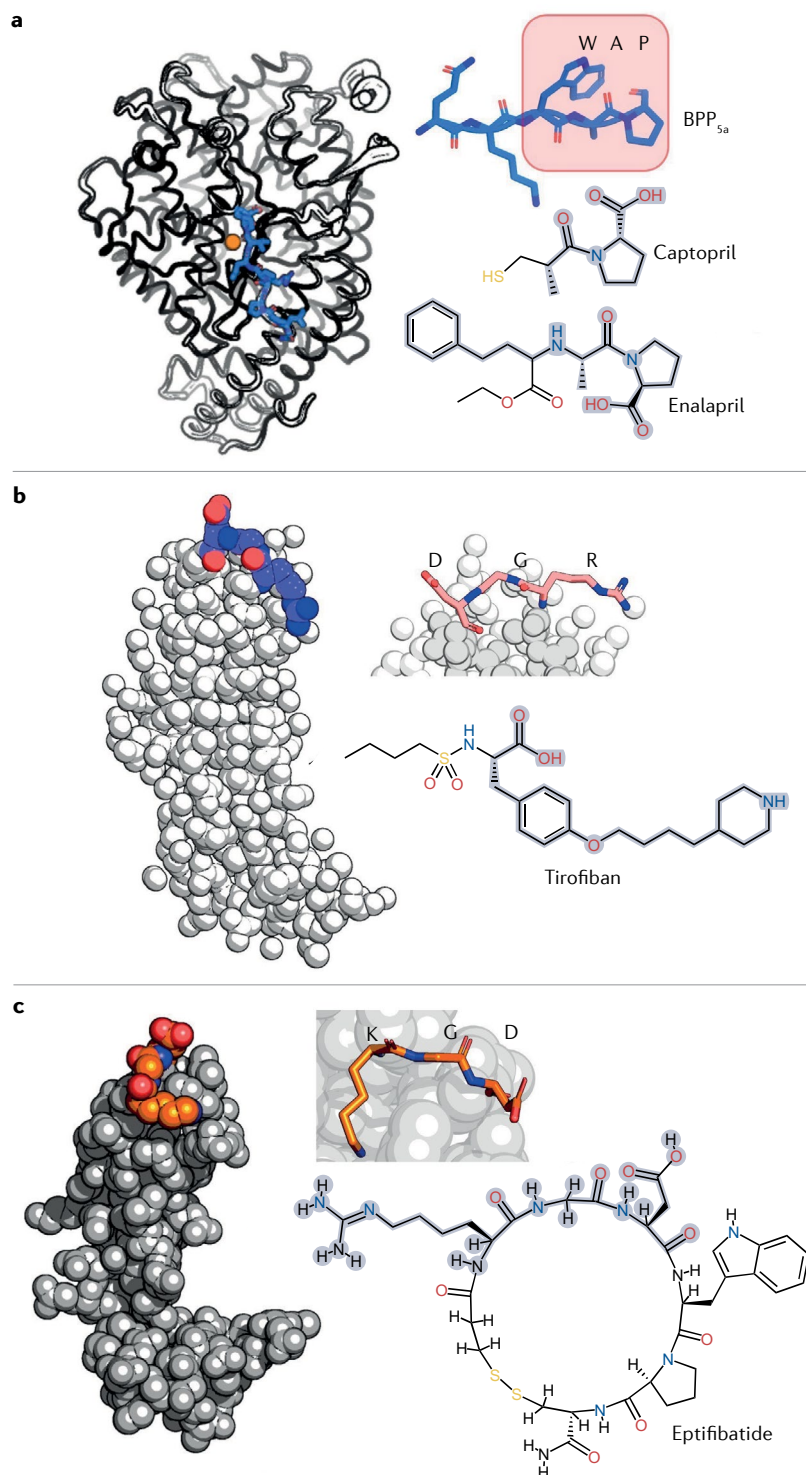


Fig. 5 | Approved drugs derived from snake venoms.

Several drugs derived from snake venoms have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The chemical structures of these drugs are shown, with the region that mimics the snake toxin highlighted in grey. **a** | Nine hypotensive bradykinin potentiating peptides (BPPs) were isolated from the venom of the jararaca viper; they inspired the design of the antihypertensive drugs captopril and enalapril. These drugs mimic the Trp–Ala–Pro (WAP) motif by which BPP_{5a} (top right) recognizes its target: the angiotensin-converting enzyme (ACE). ACE is shown on the left in a complex with BPP_{5b}, another BPP (PDB ID: 6QS1). The ACE Zn²⁺ cofactor is shown in orange.

b | The drug tirofiban was inspired by a disintegrin called echistatin found in the venom of the saw-scaled viper. Echistatin, shown on the left (PDB ID: 6LSQ), binds specifically to the $\alpha_{IIb}\beta_3$ integrin through its Arg–Gly–Asp (RGD) motif (coloured spheres and top right), which prevents platelet aggregation. In tirofiban, the piperidine moiety replicates arginine, the aliphatic linker replicates glycine, and the tyrosine carboxyl group replicates the aspartic acid carboxylate. The (S)-NHSO₂nC₄H₉ group increases the affinity of tirofiban for its $\alpha_{IIb}\beta_3$ target.

c | Eptifibatide is an antiplatelet drug inspired by the disintegrin babourin purified from the venom of Barbour's pygmy rattlesnake. A homology model of babourin (template PDB ID: 1J2L) is shown on the left. Most disintegrins recognize the $\alpha_{IIb}\beta_3$ integrin through the RGD motif, but babourin uses a Lys–Gly–Asp (KGD) motif (coloured spheres and top right). Eptifibatide achieves maximum selectivity owing to the fusion of the two motifs into the unnatural homoRGD motif. Additional peripheral residues and cyclization endow further molecular recognition capabilities and resistance to proteolysis.

Eptifibatide. Eptifibatide is another antiplatelet drug approved by the FDA in 1998 and EMA in 1999 that was developed from a disintegrin (barbourin) found in the venom of Barbour's pygmy rattlesnake (*Sistrurus miliaris barbourin*)^{107,108,114,115}. Barbourin binds the $\alpha_{IIb}\beta_3$ integrin through a Lys–Gly–Asp (KGD) motif, rather than the more common but less specific RGD motif. The KGD motif provides excellent specificity for the $\alpha_{IIb}\beta_3$ integrin over other integrins¹¹⁵.

Residues adjacent to the KGD motif greatly affect the affinity of barbourin. Therefore, these neighbouring regions were also elucidated during the development of eptifibatide^{115,116}. The final form of the drug consists of a heptapeptide cyclized through a disulfide bridge. Cyclization provides superior resistance to proteolysis^{21,116,117}. Interestingly, the motif presenting the highest affinity and specificity for the $\alpha_{IIb}\beta_3$ integrin was neither RGD nor KGD, but a 'hybrid' of these, homoRGD (FIG. 5c). This surprising result indicates that there are limits to the structural versatility of protein toxins based on a small number of genetically encoded amino acids. The optimal structural solutions for molecular recognition might not be achievable through genetically encoded amino acids only, and might instead require complex and metabolic post-translational modifications that are too expensive for a secretion that a snake frequently depletes and reproduces. The versatility of synthetic chemistry presents an advantage that can be exploited to achieve affinity and specificity beyond what is observed in nature.

motif of echistatin within a small synthetic molecule⁹². The affinity of tirofiban for $\alpha_{IIb}\beta_3$ was enhanced by the (S)-NHSO₂nC₄H₉ extension, which interacts with an $\alpha_{IIb}\beta_3$ exosite with which echistatin does not interact¹¹² (FIG. 5b). The affinity and specificity of tirofiban thus surpass those of echistatin. Tirofiban is another example of the transformation of a venom toxin into a life-saving drug. It is also one of the first documented successful pharmacophore-based drug discovery applications¹¹³.

In addition to the drugs approved by the FDA and the EMA, other snake venom toxins have been approved for clinical use in other countries and are described below.

Batroxobin. Batroxobin (Defibrase) is a thrombin-like serine protease purified from the venom of the Brazilian lancehead pit viper (*Bothrops moojeni*) that induces defibrinogenation^{4,21,118,119}. This toxin is marketed in China and Japan for the treatment of acute cerebral infarction, ischaemia caused by vascular

occlusive diseases, and peripheral and microcirculation dysfunctions.

Haemocoagulase. Haemocoagulase (Reptilase)^{21,120} is an enzyme system purified from the venom of the common lancehead pit viper (*Bothrops atrox*). The enzyme system includes batroxobin and an SVMP that activates factor X, which results in anti-haemorrhagic activity. Haemocoagulase is approved for use in Japan, India and South Korea to treat internal and external haemorrhages.

α -Cobrotoxin. α -Cobrotoxin, which is purified from the venom of the Chinese cobra (*Naja atra*)^{21,121,122}, is a 3FTx α -neurotoxin that binds nicotinic acetylcholine receptors at the neuromuscular junction. α -Cobrotoxin is approved for use in China as an analgesic for moderate to severe pain. However, its high bioactivity might lead to side effects, such as respiratory arrest.

Drugs in preclinical and clinical trials

Several compounds based on components from snake venom are in preclinical and clinical trials^{21,120,123}. We focus on selected examples that are among the most promising and advanced in preclinical or clinical trials.

Anfibatide. Anfibatide is an anticoagulant C-type lectin-like protein purified from the venom of the sharp-nosed viper (*Deinagkistrodon acutus*). The protein is heterodimeric, comprising α -subunits and β -subunits linked by seven disulfide bonds. The anticoagulant activity of anfibatide is due to its strong binding to human platelet glycoprotein Ib α -chain (GPIb α), which inhibits the binding of GPIb α with von Willebrand factor (VWF) and thrombin^{124,125} (FIG. 6a). The binding of GPIb α and VWF is key to triggering platelet adhesion and thrombosis, particularly under the high shear stress conditions at sites of arterial stenosis¹²⁶, which lead to myocardial infarction and stroke. In addition, anfibatide decreases thrombus volume and stability¹²⁴.

Recombinant anfibatide was produced at a pilot scale in yeast¹²⁷, avoiding issues relating to quality control and the limited supply of snake venom. Anfibatide might become the first drug to target GPIb α , which would be a game-changer for anticoagulant therapy, as anfibatide does not interfere with haemostasis and thus does not seem to cause the haemorrhages that currently marketed drugs do. So far, anfibatide has passed phase I clinical trials¹²⁴.

Crotamine. Another toxin with tremendous therapeutic potential is crotamine. This toxin is a small defensin purified from the venom of some populations of the South American neotropical rattlesnake (*Crotalus durissus*)¹²⁸. Although the venom is very toxic, crotamine has low myotoxicity and neurotoxicity¹²⁹. Crotamine is a very basic (with a pI of 10.3 and a charge of +8) amphipathic 42-residue peptide with three disulfide bridges and structural folds similar to those of human α -defensins and β -defensins^{60,130} (FIG. 6b).

Crotamine is a cell-penetrating peptide that is rapidly internalized into almost all cell types¹³¹. The primary cytotoxicity mechanism is accumulation in and disruption of lysosomes¹³². Crotamine has very high

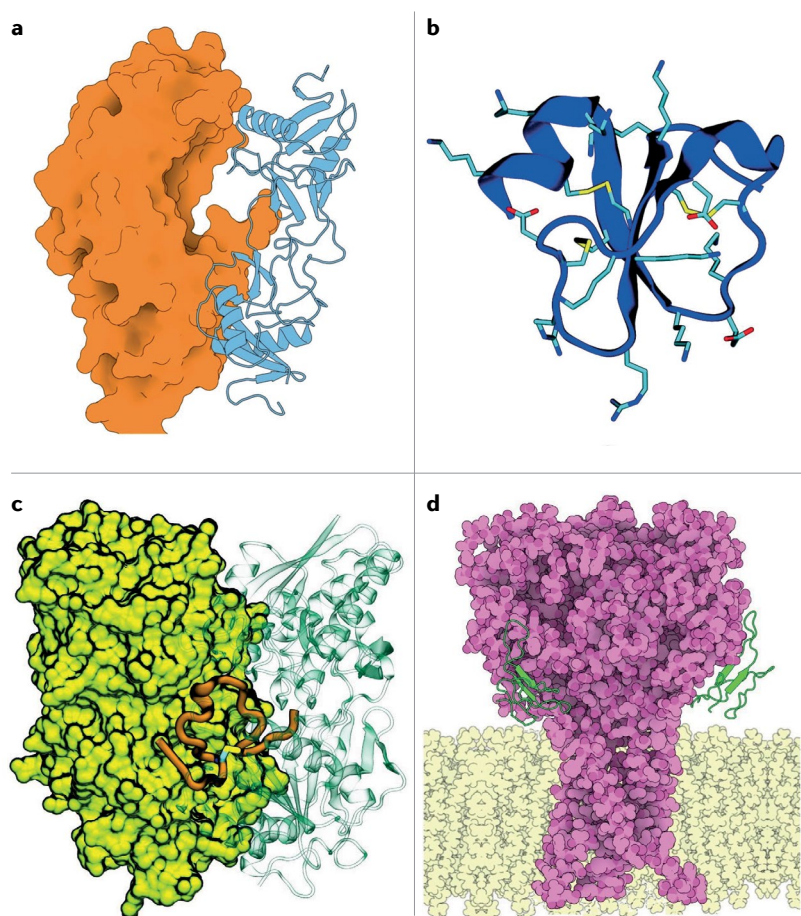


Fig. 6 | Drugs derived from snake venoms in clinical or preclinical trials. **a** | Anfibatide (blue cartoon) is a snake C-type lectin-like protein that is predicted to bind to platelet glycoprotein Ib α -chain GPIb α (orange surface)¹²⁴ at a site that partially overlaps with the GPIb α -von Willebrand factor binding surface (PDB ID: 1SQ0), thus inhibiting the association of von Willebrand factor and consequently platelet aggregation. Anfibatide is a promising anticoagulation candidate that has passed phase I clinical trials. **b** | Crotamine is an amphipathic and highly basic defensin that penetrates cells and is resistant to proteolysis. Crotamine exhibits antiproliferative, antinociceptive and analgesic activity in vivo upon oral administration. Cationic residues are shown as sticks and the disulfide bonds are shown in yellow. **c** | *Dendroaspis* natriuretic peptide (DNP) from the eastern green mamba (ochre tube with the disulfide bond in yellow) bound to the dimeric particulate guanylyl cyclase A receptor (shown as a lime surface and a green transparent cartoon) (PDB ID: 7BRI). Cenderitide is a natriuretic peptide chimaera resulting from the fusion of human C-type natriuretic peptide (CNP) to DNP and co-activates both DNP and CNP transmembrane receptors. **d** | The three-finger toxins mambalgins-1 and mambalgins-2 bind to the acid-sensing ion channels 1a and 1b, locking the channels in the closed state and impairing their function, with an analgesic effect as potent as that of morphine but with much lower toxicity in rodents. The complex of mambalgins-1 (green) with the transmembrane (light yellow) acid-sensing ion channel 1a (violet) is shown (PDB ID: 7CFT). The mambalgins are promising scaffolds for the development of a new generation of analgesics.

Natriuresis

The process of excretion of sodium in the urine.

selectivity for actively proliferating cells¹³³, such as cancer cells, making it a promising antitumour agent¹³². Its anti-melanoma activity was demonstrated in mice¹³⁴ without toxicity to healthy cells and it can even be administered orally^{129,135} owing to its excellent resistance to proteolysis and its cell-penetrating ability.

Among other bioactivities (TABLE 1), crotonamine also exhibits antinociceptive activity (and is 500 times more potent than morphine (mol mol^{-1})¹³⁶ and anti-inflammatory activity in in vivo mouse models and upon oral administration^{129,135} without toxic side effects. Furthermore, its chemical and recombinant syntheses were recently reported^{137–139}, which are fundamental steps required for upscaling crotonamine production. Thus, the therapeutic future of crotonamine looks promising.

Cenderitide. Cenderitide is a natriuretic peptide based on one purified from the venom of the eastern green mamba (*Dendroaspis angusticeps*) and is under clinical trials for the treatment of heart failure^{55,140}.

Natriuretic peptides are regulators of body fluid volume and induce natriuresis, diuresis, vasodilation and hypotension, as well as inhibiting fibrosis, among other bioactivities. Natriuresis and diuresis are essential for the treatment of heart failure¹⁴¹. Three natriuretic peptides (atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP)) are endogenous to humans. They are small peptides of 28, 32 and 22 (or 53) residues, respectively, with a highly conserved 17-residue cyclic structure (FIG. 6c). Natriuretic peptides exert their effects by activating the particulate guanylyl cyclase (pGC)-A and pGC-B transmembrane receptors. ANP and BNP activate pGC-A, whereas CNP activates pGC-B⁵⁵. Recombinant ANP and BNP were approved for use in Japan (1995) and the USA (2001), respectively, as a therapy for acute decompensated heart failure. However, later studies suggested that their efficacy is questionable^{142,143}.

Snake venoms are a rich natriuretic peptide source, as hypotension leads to a rapid loss of consciousness in their prey. One of the first natriuretic peptides discovered in snake venom was the 38-residue *Dendroaspis* natriuretic peptide (DNP) from the venom of the eastern green mamba (FIG. 6c). DNP activates pGC-A and is as potent as ANP but is more resistant to metabolic ring opening. Cenderitide is a DNP–CNP chimaera that results from the addition of the 15 C-terminal residues of DNP to the C-terminal residue of CNP. It was designed to have the ability to co-activate both pGC receptors. Clinical trials have shown that receptor co-activation gives cenderitide natriuretic, diuretic and, possibly, anti-fibrotic activities without the undesirable hypotension effect of ANP and BNP. Cenderitide is safe and well tolerated in people with stable chronic heart failure^{144,145}.

Mambalgins-1 and mambalgins-2. Mambalgins are 57-residue members of the 3FTx family purified from the venom of the black mamba that inhibit isoforms 1a and 1b of the acid-sensing ion channels (ASICs)¹⁴⁶. ASICs are Na^+ transporters activated by a decrease in the extracellular pH¹⁴⁷. These channels, expressed in nociceptive neurons, have a central role in pain pathways and other critical

pathophysiological processes, such as ischaemic strokes and tumour growth¹⁴⁸. Mambalgins inhibit ASIC1a and ASIC1b in the central and peripheral nervous systems with nanomolar affinity, both in vitro (rat and human) and in vivo (rat)^{146,149,150}. In rodents, administration of mambalgins into the peripheral or central nervous systems strongly abolishes acute and inflammatory pain^{146,151,152}, with an analgesic effect as potent as that of morphine but with much less tolerance and without the respiratory arrest typical of morphine and toxic side effects¹⁴⁶. Thus, mambalgins represent molecular scaffolds for a new generation of strong, non-toxic analgesics.

Mambalgins-1 and mambalgins-2 have been chemically synthesized and their structures determined^{149,150}. These mambalgins represent a new family of 3FTx; they share the core of a typical 3FTx but with short first and third fingers and an elongated middle finger. The structure of the human ASIC1a channel, both free and bound to mambalgins-1, was determined in 2020 (REF.¹⁵³). Experimental and computational studies¹⁵⁴ used this structure to refine earlier proposals for the working model of ASIC inhibition, that is, the locking of the channel in the closed state (FIG. 6cd). The recent wealth of activity and structural data have laid a solid foundation for the structure-based rational design of mambalgins analogues with favourable delivery routes.

Toxins targeting SARS-CoV-2 virus. We conclude this section with a review of early-stage in vitro tests of toxins that target the SARS-CoV-2 virus, which have directed considerable attention to the medicinal potential of snake venom.

Given the well documented antiviral and antimicrobial activity of snake venom PLA2s^{4,21,73,123,155,156}, the activity of eight snake venom PLA2s against SARS-CoV-2 was tested in Vero cells¹⁵⁶. The PLA2s were purified from the venoms of the banded krait (*Bungarus fasciatus*), the steppe viper (*Vipera renardi*) and the Nikolsky's viper (*Vipera nikolskii*). The most active was the heterodimeric PLA2 HDP-2 from the Nikolsky's viper, which exhibited nanomolar virucidal activity. The phospholipolytic activity of HDP-2 probably destroyed the viral envelope. HDP-2 also inhibited virus–host cell fusion. Direct interaction between the catalytically active subunit of HDP-2, HDP-2P, and the essential cellular ACE2 receptor was confirmed by surface plasmon resonance. All the tested PLA2s exhibit low cytotoxicity¹⁵⁶.

Small peptides derived from the C-terminus of the myotoxin bothropstoxin-I (KKYRYHLKPFCKK), a PLA2-like protein purified from the venom of the Brazilian pit viper jararacussu (*Bothrops jararacussu*), have shown antimicrobial activity against Gram-positive, Gram-negative and multidrug-resistant bacterial strains¹⁵⁷. Moreover, these peptides plus several analogues were tested against SARS-CoV-2 in Vero cells¹⁵⁸. Three peptide dimers showed notable activity and selectivity against SARS-CoV-2. In addition, their cytotoxicity was low. The peptides targeted the viral papain-like cysteine protease with low, micromolar potency (with a binding affinity of 0.9–7 μM). Viral papain-like cysteine protease is an attractive SARS-CoV-2 target owing to its fundamental role in the cleavage and processing of viral polyproteins¹⁵⁹.

Thus, although these studies are still in their infancy, the use of snake venom to treat SARS-CoV-2 infection is of increasing interest.

A drug repertoire from snake venom

It is evident that snake venom possesses immense therapeutic potential but that it is far from being fully exploited. This observation raises questions regarding the challenges that need to be overcome to transform snake venom into a drug repertoire.

Identifying drug candidates

Characterizing the toxins in the proteome of each species is fundamental, and this task has been facilitated by various technological advances. Snakes are the animals for which this characterization is most advanced, in part owing to a large amount of venom each individual produces compared with that of smaller animals, such as scorpions, spiders, centipedes or cone snails, whose peptide-based venoms are also promising from a therapeutic perspective^{3,4,20}. However, the chemical structural diversity found in an animal venom is frequently less vast than that of large chemical compound libraries^{160–164}, such as ZINC¹⁶², which is a widely used and ever-growing database that contains more than nine hundred million drug-like compounds. Pharmaceutical companies invest vast sums of capital in maintaining and expanding massive, private compound libraries. These infrastructures aim to fulfil a basic need of modern drug discovery: chemical diversity. In other words, if the goal is to conduct high-throughput ligand screening for a target of interest, it will not be easy to justify that the use of animal venoms is advantageous over the more extensive and more diverse traditional chemical libraries.

The advantage of animal venom toxins is their high, specific and inherent bioactivity, which enables a drug-candidate toxin to be chosen on the basis of a previously observed bioactivity and not through high-throughput screening. The selection process has to be rational, not automated. It is easy to glimpse the wealth of diseases we can eventually treat by looking at the summarized list of bioactivities reported in TABLE 1. Nevertheless, this approach implies the need for flexibility in the pharmaceutical industry, which is currently focusing on automation¹⁶⁴.

There are two paths for developing drugs from snake venoms: the use of toxins without modifications or the design of small synthetic compounds that mimic the recognition motifs of toxins, which are called toxinomimetics.

The use of unmodified snake toxins has not been very successful. Neither the FDA nor the EMA has approved batroxobin, haemocoagulase or cobrotoxin. However, the FDA and the EMA have approved unmodified peptide toxins from the venom of other animals⁴, such as bivalirudin (a toxin from the medicinal leech used to prevent coagulation during surgery)¹⁶⁵, ziconotide (a toxin from the magic cone snail used to treat chronic pain)⁹⁶ and exenatide (a toxin from the Gila monster used to treat type 2 diabetes)¹⁶⁶.

The use of unmodified toxins as prescription drugs comes at a cost: it hinders their administration, stability

and large-scale production. To circumvent these issues, toxinomimetry, a technique that involves complex, rational transformations of the toxin core, has been successful for deriving drugs from snake venoms, as shown by the development of captopril and its analogues, as well as the development of tirofiban and eptifibatide. This path is not always preferable to the use of unmodified toxins. The preferable path is simply the one that provides more efficient, safer and cheaper drugs.

Routes of administration

As most toxins are peptides or proteins, their administration is usually problematic. Despite considerable resistance to proteolysis, owing to the numerous disulfide bridges within most toxins^{34,167}, oral administration is generally inefficient, in part owing to difficulties in crossing cell membranes. This issue is evident in the lack of oral bioavailability of most snake-venom-based drugs²¹. Snake venom toxins are usually efficient *in vivo* upon parenteral injection, which is not surprising considering that they have evolved to be bioactive when injected through the snake's fangs into their predators and prey.

Intravenous administration is generally the least invasive administration route that works, constraining the appeal of toxins for drug discovery. The cost and complexity of their large-scale synthesis, extraction and purification or heterologous expression is a further constraint. Toxinomimetry is a possible way to overcome these problems by using the toxin as a reporter rather than a drug. In this strategy, the role of the toxin is to reveal the molecular determinants of activity and specificity for the target, which are then mimicked as extensively as possible with a small synthetic molecule that is affordable to synthesize and orally bioavailable.

Target identification

The realization of the full power of toxinomimetry requires the resolution of the structures of toxin–target complexes of interest. Identification of the targets of haemotoxins in the well known coagulation cascade has been quite successful¹⁶⁸; conversely, identification of the targets of neurotoxins in the less understood and more complex human neurological system has proved more challenging²⁰. Venom toxins are probably the best lead sources for drugs directed at ion channels⁴, which are central targets in modern drug discovery, accounting for almost a fifth of today's total drug targets¹⁶⁹. However, the functions of many of these channels are poorly understood, if not uncharacterized. Their molecular structures are often unknown and challenging to determine. The many isoforms of ion channels further complicate their selective targeting¹⁶⁹.

If a toxin binds an unknown target, identification of the target is challenging owing to the technical difficulty of screening target pools. For example, myotoxic PLA2s possess a region named the 'pharmacologic site' that binds a membrane protein prevalent in the sarcolemma. The binding of PLA2s to such a membrane protein narrows its biodistribution, focusing its hydrolytic action on muscle tissues^{170,171}. However, even after many years of study, the identity of the myotoxic PLA2 pharmacologic target is still unknown.

When target identification is successful, determining the target structure and the target–toxin complex is still challenging. Knowing the 3D structure of the complex is a requisite to the understanding of molecular recognition, without which the design of toxinomimetics within a structure-based paradigm is not possible. In this case, toxinomimetry has to resort to a ligand-based paradigm, supported by measurements of ligand affinity for toxin mutants, which is less efficient than structure-based drug design because it is rooted in less molecular information.

Computational chemistry in toxinomimetry

The path forward should entail, at least in part, a much deeper involvement of computational chemistry. The increase in computational power allows for the more exact implementation of physical principles, which, together with the greater involvement of deep learning and artificial intelligence, is powering advances in computational fields important for snake-venom-based chemistry and drug discovery, such as protein homology modelling^{172–177}; protein–protein docking^{178–185}; computational mutagenesis, in particular alanine scanning^{186–191}; and the determination of enzymatic mechanisms^{88–91}. Computational chemistry can thus have a decisive role in speeding up the process of drug discovery based on snake venom toxins.

Computational chemistry can intervene whenever a toxin with the bioactivity of interest acts on an unknown target. Today, it is possible to assemble a database of biological targets for which the molecular structures have been determined by X-ray or cryogenic electron microscopy and homology modelling and then to screen the database according to toxin–target affinity. In several cases, the uncertainties associated with homology modelling¹⁷² and docking¹⁷⁸ do not allow for the identification of a single and robust target. Nevertheless, computational chemistry reduces the target pool to a set small enough to be feasible for experimental testing.

It is also challenging to determine target–toxin complex geometries with atomic-level accuracy through computation alone¹⁷⁸, particularly when modelled structures are involved. Despite this, computational chemistry can narrow down the target and toxin regions that contact each other to the point at which experimental mutagenesis (and other techniques) can be applied to provide the final atomic-level information. As an example, computational and experimental methodologies were used together to clarify the mechanism by which mambalgins inhibit ASICs^{146,154}.

In summary, high-level computational chemistry has the power to advance target identification and target–toxin structural determination if conducted in synergy with experiments; together they could facilitate either the use of unmodified toxins or the modelling of toxin-based small ligands. For the latter, traditional

medicinal chemistry can be employed to reduce the toxins into small, synthetic, bioavailable molecules while keeping most of the determinants for recognition and affinity. This approach follows the ‘captopril way’, which was a lesson of success at every level in drug discovery.

Conclusion

In modern Western civilization, the snake represents deceit and triggers both fascination and fear. However, ancient civilizations respected the snake owing to the healing power of its venom. It is becoming evident that the ancients were right, as the venom of this splendid animal is an extraordinary library of bioactive compounds that has great medicinal potential.

Our survey of hundreds of studies conducted over the past 15 years reveals the diversity in the chemical composition of snake venom, which is increasingly being explored for the development of drugs. Efforts to elucidate the chemical reactivity of the principal toxins within venom is helping to increase understanding of how toxins act on their prey targets, and how one can engineer toxin action to achieve a therapeutic goal. Furthermore, understanding of venom chemistry allows for the rational design of transition-state small-molecule analogue inhibitors for primary enzymatic toxins that are today the most promising candidates for replacing the difficult-to-manage and expensive antibody-based treatments for snakebite envenoming. The molecular recognition features of snake venom toxins are also being explored at a molecular level. As we have emphasized, these recognition motifs can be mimicked by small-molecule drugs directed to the toxin’s targets, which is a promising approach for developing cheap, stable, easily scalable and orally available medicines in drug discovery.

The drugs already approved and under development derived from snake venom demonstrate that toxic bioactivity can be transformed into a therapy for the right disease. Large toxin molecules can be redesigned and reduced to their recognition motifs for oral delivery while maintaining affinity and specificity. Of the many drugs in preclinical development, mambalgins in particular reflect the contrast between their therapeutic promise (in this case, to relieve pain) and their origin from one of the most feared snakes on the planet.

In terms of the future of venom-based drug development, we assert that toxinomimetry is an exciting alternative and a complement to the use of unmodified toxins. Furthermore, computational chemistry, which is still underused in the field, can accelerate the understanding of snake venom chemistry and hence the development of new drugs. We hope that this Review will inspire a new generation of scientists to explore and realize the immense potential of snake venoms.

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This study provides insight into the molecular-level mechanism of physiological substrate recognition by SVMPS.

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Author contributions

All authors contributed to the discussion of content and edited the article prior to submission. P.A.F., A.L.O., M.F.V. and M.J.R. also researched the data and contributed to the writing of the article.

Competing interests

The authors declare no competing interests.

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