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Review Article

Therapeutic potential of snake venom: Toxin distribution and opportunities in deep learning for novel drug discovery

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ABSTRACT

Snake venom is a rich source of bioactive molecules that hold great promise for therapeutic applications. These molecules can be broadly classified into enzymes and non-enzymes, each showcasing unique medicinal properties. Noteworthy compounds such as Bradykinin Potentiating Peptides (BPP) and Three-Finger Toxins (3FTx) are showing therapeutic potential in areas like cardiovascular diseases (CVDs) and pain-relief. Meanwhile, components like snake venom metalloproteinases (SVMP), L-amino acid oxidases (LAAO), and Phospholipase A_2s (PLA_2) are paving new ways in oncology treatments. The full medicinal scope of these toxins is still emerging. In this review, we discuss drugs derived from snake venoms that address CVDs, cancer, diabetes, strokes, and pain. Further, we outline the toxin distribution across 130 snake species, categorized by their genus within the Crotalidae, Viperidae, and Elapidae families. Conclusively, we spotlight the potential of Deep Learning (DL) in discovering groundbreaking drug prospects from these toxins.

1. Introduction

Snake venom is a complex mixture of abundant components, containing a variety of biologically active molecules with peptides and proteins accounting for more than 95 % of snake venom dry weight (the other 5 % contain lipids, carbohydrates, and biogenic amines) [1]. These proteins perform various functions aiding in capturing and digestion of prey after envenomation. Some proteins function as enzymes catalyzing chemical reactions that disrupt coagulation and induce hemorrhage, while others interfere with cellular receptors, causing paralysis. The venom's effects on the body can be hemotoxic, cytotoxic, or neurotoxic, leading to blood cell damage, tissue inflammation, or nervous system disruption, respectively. The specific physiological impacts depend on the snake species and venom composition [2].

Snake venom composition can be divided into two groups of proteins and peptides: enzymatic and non-enzymatic molecules [3]. Enzymatic molecules are proteins with catalytic sites and activity, able to speed up chemical reaction rates in the presence of a substrate. The most common and abundant enzymatic molecules are snake venom phospholipases A_2 (PLA_2), snake venom metalloproteinases (SVMP), snake venom serine proteases (SVSP), and L-amino acid oxidases (LAAO) [4]. The second group is the non-enzymatic molecules found in snake venom. These include neurotoxins, affecting the nervous system; cardiotoxins, affecting the heart; and cytotoxins, damaging cells. The most common non-enzymatic molecules are three-finger toxins (3FTx), Kunitz peptides (KUN), disintegrins (DIS), and cysteine-rich secretory protein (CRiSP) [5] (Fig. 1).

Chronic diseases, including CVDs, cancer, diabetes, and chronic pain, are the leading causes of death and disability worldwide. These conditions have been treated using drugs derived from Viperidae and Elapidae venoms. For example, Captopril, used for hypertension and diabetic nephropathy, was developed from the BPP in snake venom [6,7]. DIS, peptides isolated from snake venoms and act as anti-coagulant agents, inhibiting platelet aggregation and promoting hemorrhage, resulted in the development of Eptifibatide and Tirofiban, two FDA-approved drugs for the treatment of acute coronary syndrome. Among approved venom-derived molecules for the treatment of chronic diseases and pain, we find Captopril, Enalapril, Eptifibatide, Tirofiban, Defibrase, Reptilase,

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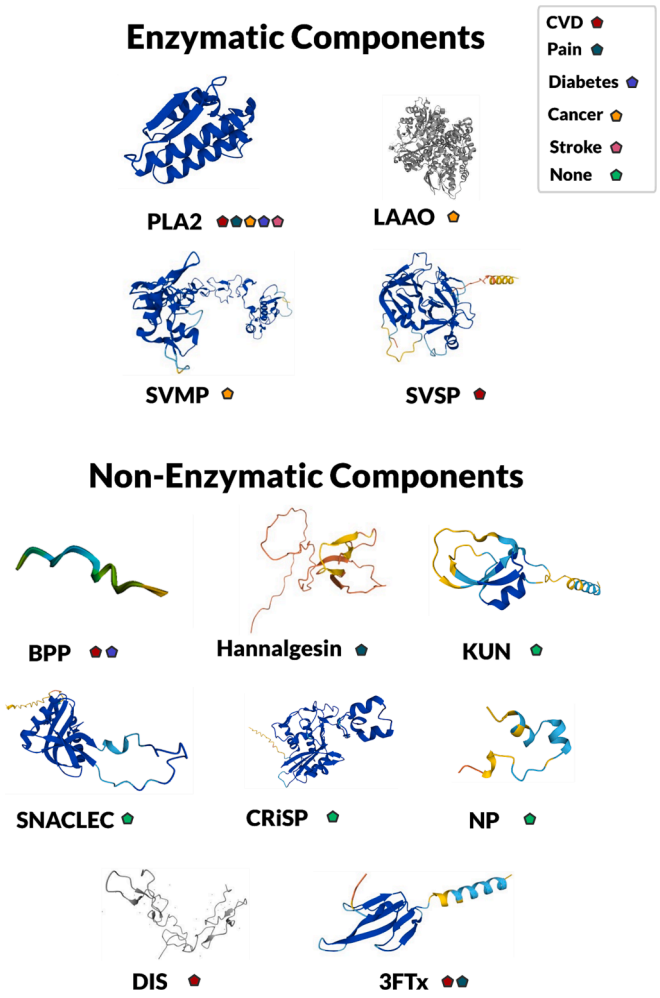

Fig. 1. 3D structures of toxins used in the treatment of chronic diseases and pain, classified into enzymatic and non-enzymatic components. Labels identify therapeutic effects of each molecule. The 3D structure of BPP was generated using Alphafold2 from its primary structure, and all other 3D structures were obtained from Uniprot [50].

and Cobratide that have either been approved by the FDA (USA), the EMA (Europe), or the National Medical Products Administration (NMPA) (China) [8–12]. Table 1 summarizes the uses of the seven approved drugs, including their UniProt ID, the identified mechanism of action, and the approval jurisdiction.

2. Snake venom composition

According to the reptile database, there are more than 3,900 snake species worldwide [45]. The Viperidae and Elapidae families contain over 700 known species of venomous snakes [45] with venoms that are either hemotoxic, neurotoxic, or cytotoxic. The Viperidae family includes rattlesnakes, copperheads, and cottonmouths, known for their hemotoxic and cytotoxic effects [46]. This family is responsible for approximately 98 % of all envenomations in the United States [47] and nearly 50 % in India [48]. The Elapidae, which include cobras, coral snakes, kraits, and mambas, are known for their neurotoxic effects, which in most cases cause paralysis [49].

Crotalinae and Viperinae are both subfamilies of the Viperidae family and present similar protein distributions (Fig. 2A) with percentages of SVMP, PLA_2, and SVSP being similar between the two subfamilies. The primary distinction between the two is the presence of 3FTx in Elapidae and its complete absence in Viperidae (Fig. 2A). One of the most noticeable similarities between the Viperidae and Elapidae families is the dominant presence of PLA_2. However, the Elapidae has a higher percentage of 3FTx (57 %), PLA_2 (33 %), and a lower percentage of SVMP (3 %) (Fig. 2A).

The proteomic composition of snake venoms varies depending on several factors, such as location, leading to differences in the dominant protein percentages among different populations of the same species. Despite these differences, all populations of *B. atrox* from Brazil, Venezuela, Peru, and Colombia have a higher percentage of SVSP, indicating their relative importance in the venom composition. Interestingly, PLA_2, SVMP, and SVSP are abundant, contributing to the venom's toxicity and other pharmacological effects (Fig. 2B).

PLA_2, SVMP, and SVSP are prevalent in most *Crotalus* species; however, the venom of *Crotalus durissus cascavella* and *C. d. collilineatus* contains a higher abundance of PLA_2 and a comparatively lower abundance of SVSP and SVMP (Fig. 2C). Additionally, the venom of *C. tigris* demonstrates a distinct profile, exhibiting the presence of only SVMP and SVSP with an absence of PLA_2. *Naja* venom contains a significant abundance of 3FTx, one of the most abundant proteins in the Elapidae family (Fig. 2D). Moreover, the presence of PLA_2, a vital protein, in *Naja* venoms underscores the complexity and diversity of its composition. The lower presence of SVMP in *Naja* venom demonstrates the unique composition of this genus compared to other members of the Elapidae family.

The percentage distribution of snake species and protein families among Viperidae, Elapidae, and Crotalinae families is depicted in (Fig. 2A). The abundances of PLA_2, SVMP, and SVSP are similar for both Viperinae and Crotalinae. Conversely, the Elapidae family is distinguished from the others by the predominance of 3FTx proteins in their venom compositions (Fig. 2D). Finally, the high abundance of PLA_2 in all families strongly suggests a primary role in toxicity.

2.1. Bradykinin Potentiating peptides (BPP)

Bradykinin Potentiating Peptides from snake venoms are a group of small peptides that range from 5 to 14 amino acids in length. Based on their structural characteristics, they can be divided into two major groups:

- Bradykinin Potentiating Peptides contain a pyroglutamyl residue at the N-terminus and a proline-rich sequence. The proline-rich sequence is not necessarily located at a specific position within the peptide. Rather, it suggests that the peptide contains a high number of proline residues compared to other amino acids. The exact position of these proline residues can vary depending on the specific BPP [51].
- Bradykinin Potentiating Peptides with a C-terminal extension beyond the proline-rich sequence [15].

The presence of proline residues is crucial for the biological activity of BPP as they confer resistance to degradation by proteases [51,52].

Bradykinin Potentiating Peptides work by inhibiting the ACE (Angiotensin-converting enzyme), a critical enzyme in the renin-angiotensin system (RAS) that converts angiotensin I (ATI) to angiotensin II (ATII) [53,54]. ATII is a potent vasoconstrictor and plays a critical role in blood pressure regulation. By inhibiting ACE, BPP effectively potentiate the action of bradykinin, leading to vasodilation and hypotension. This mechanism of action has led to the development of ACE inhibitors as a widely used class of antihypertensive drugs, with the first approved drug, captopril, being inspired by a BPP from the venom of the Brazilian viper *B. jararaca* [55,56].

Bradykinin Potentiating Peptides' primary effect is to lower blood pressure when envenoming the prey [57]. Sequencing studies of BPP highlighted the pharmacologically active Phe-Ala-Pro amino acid structure, which led to its use in developing new drugs [28]. This compound is the origin of Captopril, the first FDA-approved ACE inhibitor for the treatment of hypertension [6]. Captopril functions as an

Table 1
Approved drugs derived from snake venoms for chronic disease and pain treatments.

Chronic disease	Snake species	Proteins/ Peptides	Molecular weight (Da)	UniProt ID [13]	Proteomic identification tools	Potential applications	Approved drugs	Approval	Mechanism of action
Diabetes / CVD	*Bothrops jararaca*	BPP	1,059 Da [14]	P0C7J8	Size exclusion chromatography, RP-HPLC chromatography [15]	Treatment of hypertension, cardiac failure, diabetic nephropathy	Captopril [6] and Enalapril [16]	Captopril: FDA-approved to treat hypertension and diabetic nephropathy in 1981 [6]. Enalapril: EMA-approved in 2003 [17]	Inhibits angiotensin-converting enzyme (ACE) [18]
CVD	*Sistrurus miliarius barbouri*	DIS barbourin	7,701 Da [19]	P22827	RP-HPLC chromatography [10]	Anti-platelet agent, reduce ischemic cardiac events [20]	Eptifibatide [21]	FDA-approved in 1998 and EMA-approved in 1999 for the treatment of acute coronary syndrome [22,23]	Prevents fibrinogen and von Willebrand factor from binding to GP IIb/IIIa [21]
CVD	*Echis carinatus*	DIS echistatin	5,425 Da [19,24]	P17347	Gel filtration, cation exchange chromatography, RP-HPLC chromatography [24]	Reduce ischemic heart events [25], anti-platelet agent [26]	Tirofiban [27,28]	FDA-approved in 1998 and EMA-approved in 1999 for the treatment of acute coronary syndrome [29,30]	Inhibits GP IIb/IIIa receptors [31]
Stroke / CVD	*Bothrops moojeni*	Batroxobin	25,503 Da [32]	P04971	RP-HPLC chromatography [32]	Treatment of thrombotic diseases, anti-coagulation, inhibit stroke development [33]	Defibrase [34]	Approved for the treatment of stroke and ischemic attack in 2006 in China [35]. Approved for the treatment of deafness in Japan [36,37]	Converts plasminogen into plasmin and cleaves fibrinogen Aα-chain [38]
CVD	*Bothrops atrox*	SVSP	28,189 Da [39]	P04971	RP-HPLC chromatography [40]	Treatment of thrombotic diseases [41]	Reptilase [41]	Approved for the treatment of both internal and external haemorrhages in Japan, India, and South Korea [2].	Converts fibrinogen to fibrin and activates factor X [41]
Pain	*Naja naja atra*	3FTx	9,262 Da [42]	P60770	Sheathless capillary electrophoresis-mass spectrometry (CE-MS) [43]	Treatment of chronic pain	Cobratide [43]	Approved for managing moderate to severe pain in China in 1978[44]	Blocks the nicotinic acetylcholine receptor (nAChR)

ACE inhibitor, which is responsible for the conversion of ATI to ATII [58]. Recent studies have shown that Captopril, either alone or in combination with furosemide or hydrochlorothiazide, significantly impacts the treatment of CVDs [6,59].

Captopril also showed promise as a treatment for both type 1 and type 2 diabetes [60]. It prevents renal function deterioration in insulin-dependent diabetic nephropathy and is more effective than blood pressure control alone [61]. Its effects include reducing ATII levels, leading to vasodilation and lower blood pressure, which is particularly important for diabetic patients as high blood pressure can exacerbate kidney damage and increase cardiovascular risk [7]. Furthermore, captopril indirectly decreases aldosterone production by lowering ATII levels. Aldosterone, a hormone responsible for sodium and water retention in the kidneys, contributes to increased blood volume and blood pressure [54]. Consequently, captopril's ability to reduce both ATII and aldosterone levels contributes to better blood pressure management and kidney protection in diabetic patients [61,62].

2.2. Secreted Phospholipase A_2 (PLA_2)

Secreted Phospholipase A2 are a heterogeneous family of enzymes that play a significant role in the toxicity of snake venoms. These enzymes share a conserved structural scaffold, with a molecular weight of 13–19 kDa, 5–8 disulfide bridges, and an ability to form dimers in aqueous environments [63]. PLA_2 exert their toxic effects by specifically catalyzing the hydrolysis of the *sn*-2 ester bond in glycerophospholipids, resulting in the release of fatty acids and lysophospholipids [64]. The mechanisms of action of PLA_2 depend on the specific group of enzymes and their target sites. Group I PLA_2 are primarily neurotoxic, acting on neuromuscular junctions and causing paralysis. In contrast, Group II PLA_2 exhibit a wide range of pharmacological effects, such as myotoxicity, anti-coagulant activity, and local tissue damage [65,66].

Studies have shown that BnSP-6, a Lys-49 PLA_2 isolated from the venom of *B. pauloensis*, has anti-tumoral effects on human breast cancer cell line MDA-MB-231, killing cells through a mechanism that combines apoptotic and autophagic components [67]. Another study showed that Asp-49 PLA_2 from the venom of *B. jararacussu* has anti-tumor and anti-metastatic effects on the same cell line suggesting that this protein may be used for drug development for the treatment of breast cancer [68]. Additionally, CC-PLA2-1 and CC-PLA2-2, isolated from the venom of *Cerastes cerastes* (Horned desert viper), have been shown to effectively inhibit tumor cell adhesion and migration by interfering with α5β1 and αv integrins, resulting in angiogenesis blocking [69].

Studies have been conducted to investigate the use of PLA_2 enzymes derived from the venom of *N. nigricollis* in treating diabetes. Result suggests hypoglycemic effects, lowering blood sugar levels in diabetes patients [70]. Furthermore, PLA_2 from *N. nigricollis* venom (in two isoforms) increased insulin secretion in BRIN-BD11 cells [71–74]. This

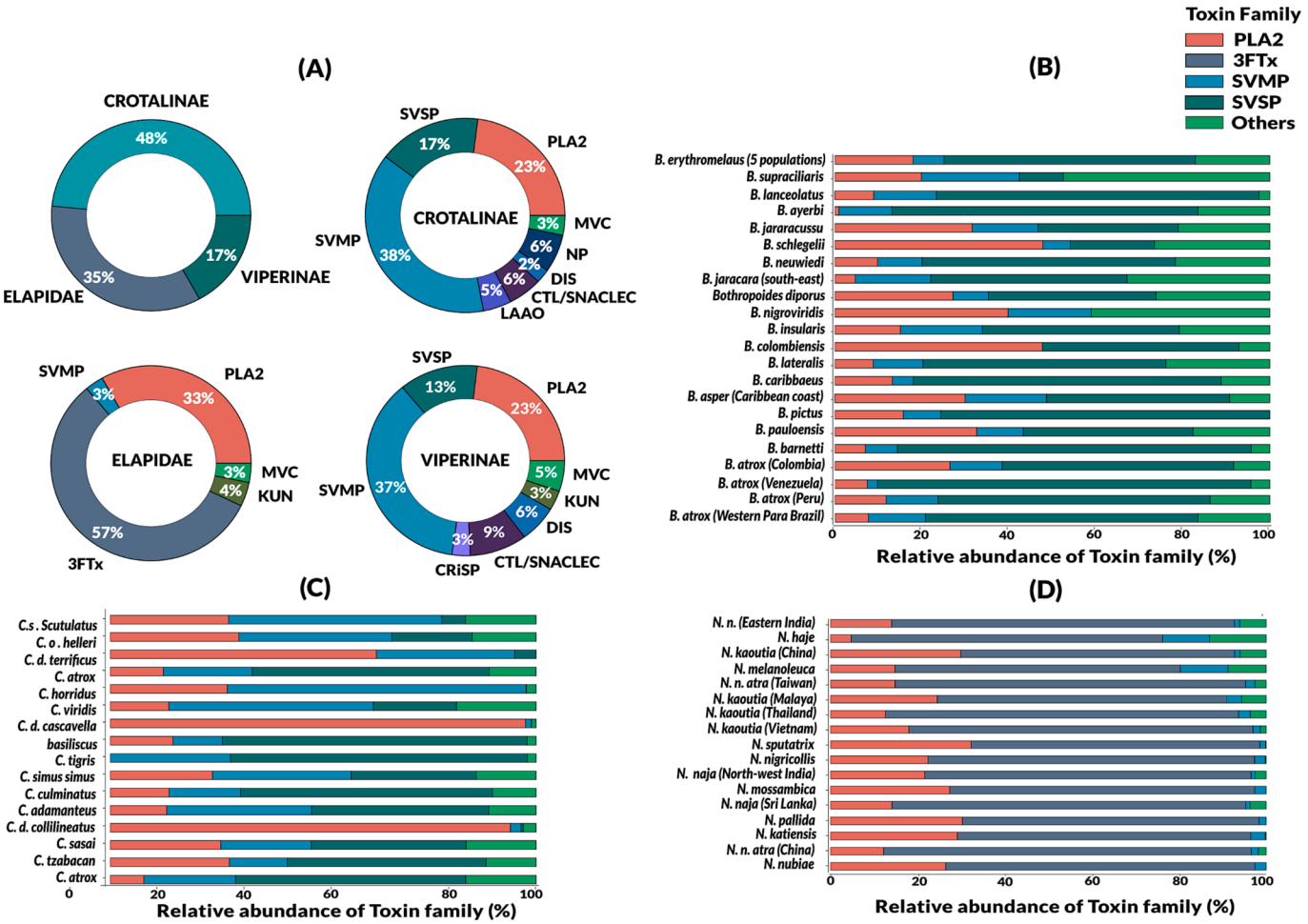

Fig. 2. Relative protein distribution in three snake genera and subfamilies: (A) Distribution of dominant protein families in Crotalinae, Viperinae, and Elapidae subfamilies; (B-D) Comparative analysis of the predominant proteins in the genera Bothrops, Crotalus, and Naja from the Viperidae and Elapidae families. Proteins analyzed are 3FTx, SVMP, SVSP, PLA_2, LAAO (L-amino acid oxidases), NP (natriuretic peptides), KUN, DIS, CTL/SNACLEC (C-type lectins and CTL-like proteins), MVC (minor venom components), and CRiSP (cysteine-rich secretory proteins).

study suggests that PLA_2 may be effective as diabetes treatments, especially for type 2 diabetes mellitus (T2DM) [73].

Acute Ischemic Stroke, which makes up nearly 90 % of all strokes and is a leading cause of death and disability globally, can be averted by inhibiting platelet aggregation to prevent blood clot formation. *N. nigricollis* venom contains PLA_2 that can impede blood clotting by inhibiting the prothrombinase complex and the extrinsic tenase complex (TF-FVIIa). BJ-PLA_2, a subtype of PLA_2 from *B. jararaca*, can block platelet aggregation, thus reducing the risk of acute thrombotic events. This suggests a potential for PLA_2 in preventing and treating CVDs.

Crotoxin is a pre-synaptic β-neurotoxin isolated from the venom of *C. d. terrificus,* known as the South American rattlesnake [75]. Crotoxin is comprised of two main components: enzymatic basic PLA_2 and crotapotin, which is a non-enzymatic acidic counterpart of crotoxin PLA_2 [76]. Crotoxin regulates pain and disease progression in an experimental autoimmune encephalomyelitis (EAE) model induced by myelin oligodendrocyte glycoprotein (MOG), a minor component of CNS myelin [77]. Crotoxin has been shown to alleviate pain in patients with advanced cancer [78–80] and has been proposed as a potential treatment for different pain conditions, including chronic neuropathic pain and cancer pain [81].

2.3. Three Finger toxins (3FTx)

Three Finger Toxins are non-enzymatic proteins with 60–75 amino acid residues and possess a molecular weight ranging from 6 to 8 kDa [82]. Structurally, 3FTx exhibit a unique fold characterized by three β-stranded loops extending from a central core, which is stabilized by 4–5 conserved disulfide bridges. This three-fingered arrangement grants the toxins their name and confers specificity in interacting with various molecular targets [83]. Found most abundantly in the Elapidae family, they are known for their neurotoxic effects caused by the postsynaptic binding of 3FTx to acetylcholine receptors (AChRs) at neuromuscular junctions [83,84].

3FTx work by primarily modulating the activity of ion channels, receptors, and enzymes within the target organism. Nicotinic acetylcholine receptors (nAChRs), muscarinic acetylcholine receptors (mAChRs), and L-type calcium channels are prominent targets. By binding to these targets, 3FTx can lead to paralysis, impaired neurotransmission, or other physiological disruptions, contributing to the venom's toxicity [85].

3FTx, isolated from the venom of *N. kaouthia*, known as the Indian cobra, was found to inhibit platelet aggregation by inhibiting ADP, thrombin, and arachidonic acid, all of which induce platelet aggregation [86]. Those isolated from the venom of *Hemachatus haemachatus*, known as the ring-necked spitting cobra, form the hemextin AB complex, which inhibits clot initiation and factor VIIa activity [87]. 3FTx' potential antiplatelet and anti-coagulant activities make them a promising target for the development of future cardiovascular drugs.

Cobrotoxin, a subtype of 3FTx, is a short-chain post-synaptic

α-neurotoxin that exhibits a high affinity for nAChRs at neuromuscular junctions [88]. This neurotoxin is primarily isolated from the venom of *N.n. atra*. The binding of cobrotoxin to nAChRs has been postulated to increase acetylcholine levels in the body by inhibiting acetylcholinesterase, an enzyme responsible for the hydrolysis of acetylcholine. As a result, pain signals may be inhibited, leading to a reduction in pain perception [89]. One such example is Keluoqu, a drug formulation containing cobrotoxin, which has been used for the treatment of chronic cancer pain. Clinical investigations have revealed that Keluoqu may be effective in alleviating moderate to severe cancer-related pain, thereby improving the quality of life for patients [89].

Cobratoxin, a subtype of 3FTx, is a long-chain post-synaptic α-neurotoxin. It was isolated from the venom of *N. n. kaouthia*, known as the Thailand cobra. Cobratoxin works by inhibiting the action of a protein called synaptobrevin, which releases neurotransmitters in the nervous system. This inhibition can lead to the suppression of pain signals. Thus, Cobratoxin has been shown to have analgesic effect, and its use as a painkiller is being investigated [90].

2.4. Disintegrins (DIS)

Disintegrins are a family of small non-enzymatic, cysteine-rich, and RGD (Arg-Gly-Asp)-containing proteins found mostly in the venom of Viperidae family. They are primarily known for their ability to inhibit integrins, which are cell adhesion receptors involved in various biological processes such as cell migration, proliferation, and survival [91].

Disintegrins cause hemorrhage by inhibiting platelet aggregation and induce apoptosis and necrosis in tissues they come in contact with [92]. They have been leveraged in developing thrombolytic drugs to prevent blood clots [27]. The FDA-approved anti-platelet drug Tirofiban (Aggrastat®) was developed from the DIS echistatin found in the venom of the viper *E. carinatus* [27]. Eptifibatide, derived from the DIS of viper *S. m. barbouri*, is also an anti-platelet drug (Integrillin®) [22]. Eptifibatide and Tirofiban, derived from snake venoms, act by blocking the binding of fibrinogen and von Willebrand factor to glycoprotein IIb/IIIa receptor on the platelet surface, thus inhibiting platelet aggregation [20].

Integrins are transmembrane receptors that have been shown to play a significant role in cancer progression and metastasis [93]. Integrins are divided into two subunits, α and β [94]. Many DIS have been shown to bind to numerous integrins, such as α2β1, which are expressed by a wide variety of cells, namely those involved in tumor development [91]. An RGD-DIS isolated from the *Porthidium lansbergii*, also known as Lansberg's hognosed pitviper, inhibited the adhesion and migration of MCF7 and MDA-DB 231 breast cancer cells by binding to integrins α2 and/or β1 [95]. Moreover, the RGD-DIS DisBa-01 isolated from the *B. alternatus* binds to αvβ3 integrin, which could prevent cell migration and adhesion in breast tumor cell 4T1BM2 [96,97].

Disintegrins have shown great promise in various diagnostic and therapeutic applications, including stroke treatment. Rhodostomin, a DIS purified from the *Calloselasma rhodostoma* venom and known as the Malayan Pit Viper, could aid in the treatment of arterial ischemic stroke [98]. Many studies strongly suggest that DIS, which act as αIIbβ3 antagonists, have anti-thrombotic therapy potential [99–101]. Nevertheless, the use of αIIbβ3 antagonists for stroke treatment is still being investigated [100].

2.5. Snake venom serine proteases (SVSP)

Snake Venom Serine Proteases are a class of proteolytic enzymes, possess a molecular weight ranging between 20 and 35 kDa and are comprised of approximately 180 to 240 amino acids, including a highly conserved catalytic triad of histidine, aspartate, and serine residues [102,103]. Structurally, SVSP adopt a globular fold similar to other serine proteases, featuring two β-barrel domains connected by a stretch of polypeptide chain, with the catalytic triad positioned at the interface of these two domains [104].

Snake Venom Serine Proteases work by hydrolyzing specific peptide bonds in target proteins, such as those involved in blood coagulation, platelet aggregation, and fibrinolysis. The serine residue in the catalytic triad acts as a nucleophile, attacking the peptide bond's carbonyl carbon to form a tetrahedral intermediate, which collapses and releases the cleaved peptide fragments [105].

Batroxobin, a SVSP derived from the venom of *B. atrox*, is a single-chain glycoprotein composed of approximately 255 amino acids. It exhibits a conserved fold characteristic of serine proteases, with two β-barrel domains connected by a single peptide chain [106]. The active site of batroxobin contains a catalytic triad comprising histidine, aspartate, and serine residues, which confer its proteolytic activity. Batroxobin exerts its action by selectively cleaving the Aα chain of fibrinogen, a key blood clotting protein, at Arg16-Gly17 peptide bond. This cleavage event results in the generation of fibrin monomers, which then spontaneously polymerize to form a fibrin clot, ultimately promoting hemostasis [2,37,102]. Studies have shown that batroxobin could decrease stroke recurrence rates and substantially improve neurological analyses [107,108]. A study using 1 h of Transcranial Doppler (TCD) Monitoring in patients with acute stroke found that batroxobin treatment can aid in neurological function recovery and reduce the risk of advancing stroke [107]. Batroxobin has demonstrated excellent results in stroke treatment when used alone or in combination with other molecules [109,110].

The potential applications of SVSP beyond hemostasis, considering intrinsic pathways involved in the wide range of biological activities of these molecules, may benefit from expanding their beneficial applications. It has been reported, a SVSP called collinein-1, derived from *C. durissus collilineatus* venom, blocking ion channels activity. SVSP toxin blocks hEAG1, a channel that plays a role in proliferation, migration, and apoptosis and contributes to cancer progression. Research into new specific inhibitors of hEAG1 channels could lead to controlling cell proliferation. Additionally, these drugs can be used in conjunction with chemotherapy to increase the patient's survival rate [102,111,112]. Another example is thrombin-like toxin called gyroxin toxin is found in the *C. d. terrificus* venom. This toxin displays hemostatic effects but also causes the "Gyroxin syndrome", a series of aberrant motor behavior in mice, suggesting SVSP can affect the blood brain barrier [113].

Ancrod, another enzymatic protein purified from *C. rhodostoma*, has previously shown great promise in treating strokes [114–116]. However, studies found that Ancrod did not improve outcomes for stroke patients compared to a placebo [117,118]. Ancrod's mechanism of action is unclear, and studies have cast doubts on its efficacy as a treatment for stroke [119].

2.6. Snake venom metalloproteinases (SVMP)

Snake Venom Metalloproteinases are Zn^{2+}-dependent enzymes found in high abundance in the Viperidae snake family. The molecular sizes of these enzymes range from 20 to 100 kDa, with amino acid sequences ranging from 200 to 600 residues [120,121]. Based on their domain composition, SVMP can be classified into three main classes (P-I, P-II, and P-III), with P-III being the most complex, containing multiple additional domains. SVMP' mechanism of action is generally characterized by the hydrolysis of extracellular matrix components such as collagen and laminin, resulting in local tissue damage and hemorrhage [96,122].

P-I SVMP class is the simplest, comprising just the metalloproteinase domain. This classification exhibits the smallest molecular sizes within the range, typically clustering around 20 kDa. Despite their relative simplicity, they can exert significant local tissue damage due to their hydrolytic action on extracellular matrix components, particularly on collagen and laminin. Their damaging activity is mostly localized, contributing predominantly to hemorrhagic outcomes [123].

P-II SVMP contain both a metalloproteinase domain and a DIS domain. These dual-domain proteins have a slightly larger molecular size compared to P-I, generally ranging from 30 to 60 kDa. The inclusion of a DIS domain augments their biological activity; the DIS domain is known for its interaction with integrin receptors, playing a significant role in inhibiting platelet aggregation. Hence, P-II SVMP not only induce local tissue damage and hemorrhage but also modulate blood coagulation, leading to an anti-coagulant effect [124].

The most complex class is P-III SVMP, which contain the metalloproteinase domain, the DIS-like domain, and a cysteine-rich domain. With this broad array of functional domains, their molecular size is correspondingly larger, usually spanning between 60 and 100 kDa. The presence of multiple additional domains endows P-III SVMP with a wide variety of biological activities, encompassing not only the functions of P-I and P-II classes but also additional effects. These may include activation of complement, inducing inflammation, and interfering with the immune response. Consequently, P-III SVMP can provoke systemic responses, resulting in more severe and multifaceted clinical manifestations upon envenomation [125].

Jararhagin, a P-III SVMP isolated from *B. jararaca*, has been shown in an in vivo treatment to reduce the incidence of nodules, metastasis, and antiproliferative inhibition capacity, and has been proposed as a potential anti-neoplastic drug [126,127].

2.7. L-amino acid oxidases (LAAO)

L-amino acid oxidases are a class of flavoproteins found in various sources, including snake venoms, and have garnered significant interest due to their multifaceted pharmacological properties. Structurally, LAAO are homodimeric proteins with a molecular weight of about 60–70 kDa per monomer and a non-covalently bound flavin adenine dinucleotide (FAD) as a prosthetic group [128]. LAAO have two domains that work together to facilitate the enzyme's catalytic activity: a FAD-binding domain and a substrate-binding domain.

L-amino acid oxidases mechanism of action involves the stereospecific oxidation of L-amino acids, converting them into their corresponding α-keto acids, along with the concomitant production of hydrogen peroxide (H_2O_2) and ammonia [129]. This enzymatic activity has been implicated in cytotoxicity, apoptosis induction, edema formation, and anti-parasitic, anti-microbial, and anti-viral activities [130–132].

The cytotoxic effects of LAAO are primarily attributed to the generation of H_2O_2, which can cause oxidative stress, leading to cellular damage and apoptosis. Furthermore, LAAO have been reported to exhibit selective cytotoxicity towards certain cancer cells, making them potential candidates for the development of novel anti-cancer therapies [133].

The LAAO isolated from the South American rattlesnake, *Crotalus durissus terrificus*, showed anti-tumor activity in several cancer cell lines [134]. Moreover, the LAAO extracted from *Ophiophagus hannah*, known as the king cobra, has been shown to have potent anti-proliferative properties against both human breast and lung cancer cells [135].

2.8. Other promising snake venom proteins and peptides

Hannalgesin is a long neurotoxin, analgesic compound derived from the venom of *O. hannah*. It has demonstrated substantial pain-relieving capabilities in preclinical studies. The mechanism of action involves an interruption in the activity of nitric oxide synthase in neurons, leading to a reduction in nitric oxide production, a known player in pain perception [136]. It also appears to interact with both the opioid and nitric oxide systems in its pain-alleviating action, suggesting a complex mode of activity [137]. Hannalgesin, exhibits analgesic activity in mice being 2,700 times more effective on a molar basis than morphine [137].

Kunitz peptides are small proteins comprised of roughly 60 amino acid residues organized in a unique α + β domain architecture, featuring two β-strands and two short α-helices [138]. This domain is rich in cysteines and is stabilized by three conserved disulfide bridges. The molecular weight of these peptides is around 6 kDa [139]. KUN have been implicated in blood clotting, fibrinolysis, response to injury or infection, and the regulation of ion channels [140]. They are primarily known as serine protease inhibitors [141].

Cysteine-rich secretory proteins are a group of non-enzymatic proteins characterized by a unique molecular structure: an N-terminal pathogenesis-related (PR-1) domain and a C-terminal cysteine-rich domain (CRD), linked by a hinge region [142]. This structure, having a molecular weight ranging between 20 and 30 kDa, is highly conserved across various snake species [143]. The mechanism of action of CRISP primarily involves blocking ion channels, particularly those associated with smooth muscle contraction and glandular secretion [144]. This leads to a variety of physiological effects, including paralysis, hypotension, and inhibition of platelet aggregation.

Natriuretic peptides (NP) are non-enzymatic peptides with a length of 25–30 amino acids and a size of 2–3 kDa, with their structures stabilized by intra-chain disulfide bonds [145]. These peptides primarily interact with natriuretic peptide receptors (NPR), including NPR-A and NPR-B, found on the surfaces of cells in various tissues. When these receptors bind, they initiate intracellular signaling cascades involving cyclic guanosine monophosphate (cGMP) [146]. This activation process then results in a series of physiological changes, including vasodilation.

Snake venom C-type lectin-like proteins (SNACLEC), are a group of non-enzymatic proteins found in snake venoms. These proteins typically consist of 120–140 amino acids and exhibit a common carbohydrate-recognition domain (CRD) of approximately 130 residues folded into a characteristic "double-loop" structure stabilized by two disulfide bridges [147]. They function primarily by interacting with specific receptors on the cell surface, disrupting hemostasis and immune responses, often leading to systemic effects like edema, hemorrhage, and necrosis [148].

3. Future perspectives on deep learning application in snake venom for drug discovery

Recent advancements in venomics have been made using Artificial Intelligence (AI). The AI Haemorrhage Analysis (AHA) tool, designed for quantifying venom-induced hemorrhage in mice, especially in snakebite scenarios, exemplifies this progress [149]. AHA can automatically identify hemorrhagic lesions using Machine Leaning (ML) methods, adjust for lighting variations, and compute Hemorrhagic Units (HaU). Its main benefits include a significant reduction in analysis time, a web interface for global laboratory use, and improved antivenom efficacy assessment. AHA stands out for its reliability, accessibility, and reproducibility, marking a notable contribution to toxinology research.

Another innovative tool is the Venom Induced Dermonecrosis Analysis tool (VIDAL) [150]. Using ML, VIDAL assesses dermonecrosis in snakebite envenomations through in vivo mouse models. It automates lesion detection, corrects for lighting inconsistencies, and measures severity with a unique Dermonecrotic Unit (DnU). VIDAL's accuracy and reproducibility are on par with histopathological analysis, making it valuable in developing treatments for this neglected tropical disease.

Furthermore, a recent systematic review focusing on AI in snakebite identification analyzed 26 studies [151]. The findings indicate that machine learning and deep learning algorithms have achieved 72–98 % accuracy in snake image classification, 80–100 % in wound image classification, and 71–97 % in other related areas. This highlights the ongoing need for better data quality and decision support systems to enhance snakebite treatment.

Recently, major developments have been realized in DL for molecular discovery (Fig. 3). Molecular contrastive learning (MolCLR) has shown significant improvements in performance on molecular property benchmarks according to recent studies [152,153]. A recent study introduces MolCLR, a self-supervised framework using Graph Neural Networks (GNNs) for molecular property prediction, overcoming

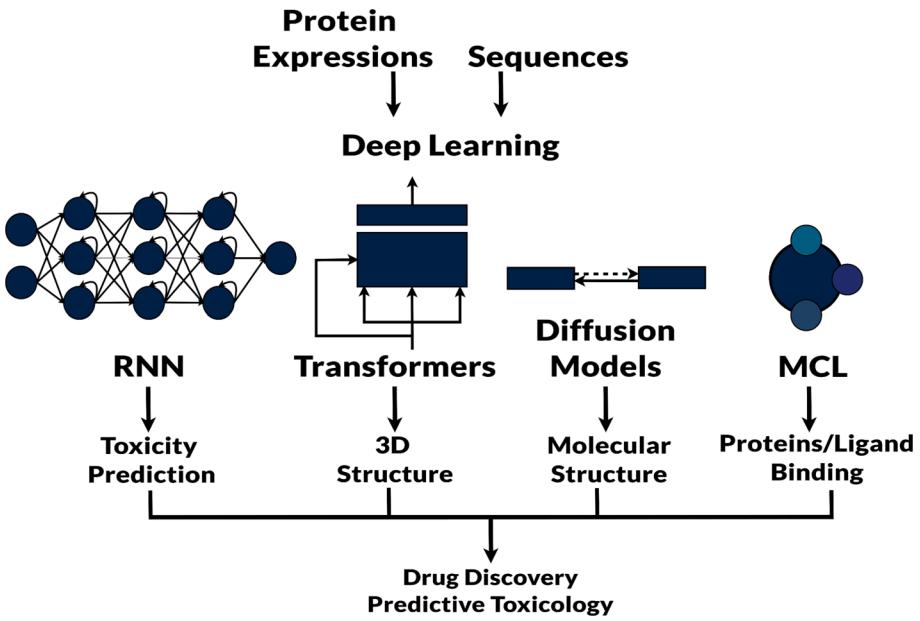

Fig. 3. Potential DL applications in venomics. DL models have been successfully applied to many aspects of drugs and molecular discovery. Several types of DL models could be applied to venomics. RNN and transformers can identify long and short-ranging patterns and molecular sequences. Diffusion models are generative networks that can generate molecular structures. MCL is a special type of training that learns to identify differences between molecules.

challenges of limited labeled data in drug discovery [154]. Using around 10 million unique unlabeled molecules, MolCLR uses innovative graph augmentations (atom masking, bond deletion, and subgraph removal) and contrastive learning, significantly boosting GNN performance on various benchmarks, achieving top results post fine-tuning, and effectively discerning molecular similarities.

This technology has potential applications in areas like snake venom-based drug discovery where it could be instrumental in developing drugs that target and inhibit snake venom toxins' receptors. The method itself involves building molecule graphs and using augmentations and a contrastive estimator. The aim is to increase the similarity between augmentations from the same molecule while decreasing the similarity between different molecules. It operates on a large set of unlabeled molecular data, aiming to boost the performance of graph neural network encoders. The underlying principle of this method is to train the model to efficiently identify and differentiate between various molecules, capitalizing on the concept of self-supervised learning where the data itself provides supervision.

Transformer-based models, like AlphaFold2, are DL models that use self-attention to process sequential data, such as protein sequences [155]. Self-attention, also referred to as the attention mechanism, enables the model to weigh and prioritize different parts of the input sequence differently. This allows it to focus more on relevant parts and less on irrelevant ones, thereby capturing long-range dependencies and intricate patterns within the data [156]. AlphaFold2 is a protein structure prediction system that uses a deep neural network based on transformer architecture to predict the 3D structure of a protein from its amino acid sequence [157]. Models such as Alphafold2 can be applied to predict the 3D structure of snake venom proteins. Such applications could, for example, help accelerate the design of new antivenoms inhibiting the effects of snake venom toxins, for instance, single-molecule antibodies structurally designed to bind to the SVMP or SVSP catalytic sites. Applications in 3D modeling would also facilitate the development of toxins as therapeutics, able to mimic the desired activity of the toxins without the associated toxicity or to mitigate "off-target" effects. A recent study evaluated AlphaFold2 and ColabFold's performance on more than a thousand snake venom toxins [157–159]. The results indicated that while caution must be exercised with proteins that have limited reference data, these tools significantly contribute to understanding protein functions and their potential applications. This implies that despite certain limitations, such models are proving to be valuable in toxinology and related fields, offering new perspectives on protein structure and function.

A recent study proposed a DL method that uses data augmentation to predict novel spider neurotoxic peptides [160]. The method combines a generative adversarial network (GAN) with Convolutional Neural Networks (CNN) to predict the neurotoxicity of virtual peptides generated by the GAN. Data augmentation methods were applied to the training dataset of known neurotoxic spider peptides to increase the data's diversity and improve the CNN's performance. The resulting model was able to accurately predict the neurotoxicity of virtual peptides generated by the GAN, demonstrating the potential of this method for discovering novel spider neurotoxic peptides [160]. (Perpetuo, L et al.) discusses the use of Artificial Intelligence (AI) in peptidomics for developing therapeutic peptides, crucial biomarkers and treatment agents for various diseases [161]. It focuses on data-driven AI methods like support vector machines, random forests, and deep learning for efficient peptide-based drug discovery. It also emphasizes AI's role in advancing peptidomics and selective peptide therapies, highlighting its importance in the prediction and development of successful peptide-based drugs.

Generative models are a class of Machine Learning (ML) algorithms aiming to learn the true data distribution of the training set to generate new data points with some variations. They have found notable applications in the field of molecular discovery [162,163]. Variational Autoencoders (VAEs) are a type of generative model used for unsupervised learning tasks. It combines an autoencoder, a neural network trained to reconstruct its input, and Bayesian variational approximation, a method for approximating complex probability distributions. A recent study proposed a DL framework that uses CNNs, VAEs, and attention mechanisms to predict drug-protein interactions using VAEs [164,165]. VAEs can be used in a variety of ways for venom-based drug discovery. For example, generative models can be trained on a known compound dataset and then used to generate new, chemically valid compounds with similar properties to the training set. This could be used to generate new leads for snake venom-based drug discovery. VAEs can also be trained on a dataset of known protein–ligand complexes and then used to predict the binding affinity of new compounds for a given protein target [166]. This can be used for the virtual screening of large venomics

datasets of compounds to identify potential hits for further development.

Another type of DL that could hold potential in the exploration and interpretation of snake venom compounds is Recurrent Neural Networks (RNNs). RNNs are comparable to transformer-based models in their ability to identify and leverage extensive, long-range patterns within biological sequences. Specifically, Bidirectional Long Short-Term Memory (BiLSTM) networks, a distinct subclass of RNNs, have demonstrated versatility across a range of applications, with drug discovery being a notable example [166–169]. "DeepLPI" is a novel DL-based model for predicting protein–ligand interactions, which can be used in drug repurposing [170]. The model uses a BiLSTM network to analyze the molecular structures of proteins and ligands to predict their interactions. The model was trained and tested on a large dataset and was found to have high accuracy and good performance compared to other existing methods [170]. These results suggest that BiLSTM models could also be applied to snake venom protein–ligand binding affinity prediction.

4. Conclusions

Snake venoms' multivalent nature, with hemotoxic, cytotoxic, and neurotoxic effects, makes it a potential source for drug discovery and development. The therapeutic potential of these venoms is fundamentally linked to their composition, the specific snake species, and the interplay of toxins present within the venom.

The integration of AI methods in venomics research has the potential to revolutionize the field of venom-based drug discovery. As venomics expands, using AI can aid in discovering new potential drugs and exploring venom evolution across various venomous species like snakes, spiders, scorpions, bees, and cone snails. This can also forecast the emergence of new venom components.

The use of antivenoms as a therapeutic intervention for venomous bites has been widely acknowledged for its efficacy in neutralizing the toxic effects of venom [171,172]. However, using antivenoms, if available due to global shortages, has serious limitations, such as efficacy and impurities can cause anaphylactic shock or serum sickness and can be costly [173]. Interestingly, toxicity prediction using ML and DL models can advance next-generation antivenoms by supporting the design of single antibodies, bioengineered antibodies, and structure-guided antivenoms against toxins. DL models can predict toxicity scores and lethal dose 50 s (LD50) by analyzing massive amounts of venomics data, which can help develop more effective and specific antivenoms. DL models can also be used to analyze blood samples from snakebite-envenomed patients to identify biomarkers indicative of the presence of specific toxins and thus allow for accurate antivenom selection and dosage needed for toxin neutralization.

Finally, 130 species represent only approximately 15 % of all venomous snake species. More efforts are needed to characterize snake venom composition across species and within the same species. To be effective these data-gathering studies should encompass different levels of biological information, including transcriptomics, proteomics, and metabolomics [174].

CRediT authorship contribution statement

Anas Bedraoui: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Montamas Suntravat:** Writing – review & editing, Data curation. **Salim El Mejjad:** Visualization, Software, Formal analysis. **Salwa Enezari:** Visualization, Software, Formal analysis. **Naoual Oukkache:** Writing – review & editing. **Elda E. Sanchez:** Writing – review & editing, Validation, Resources, Data curation. **Jacob A. Galan:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rachid El Fatimy:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Tariq Daouda:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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