REVIEW OPEN ACCESS

Journal of
Venomous Animals and Toxins
including Tropical Diseases

ISSN 1678-9199

www.jvat.org

Snake venom disintegrins update: insights about new findings

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Abstract

Snake venom disintegrins are low molecular weight, non-enzymatic proteins rich in cysteine, present in the venom of snakes from the families Viperidae, Crotalidae, Atractaspididae, Elapidae, and Colubridae. This family of proteins originated in venom through the proteolytic processing of metalloproteinases (SVMPs), which, in turn, evolved from a gene encoding an A Disintegrin And Metalloprotease (ADAM) molecule. Disintegrins have a recognition motif for integrins in their structure, allowing interaction with these transmembrane adhesion receptors and preventing their binding to proteins in the extracellular matrix and other cells. This interaction gives disintegrins their wide range of biological functions, including inhibition of platelet aggregation and antitumor activity. As a result, many studies have been conducted in an attempt to use these natural compounds as a basis for developing therapies for the treatment of various diseases. Furthermore, the FDA has approved Tirofiban and Eptifibatide as antiplatelet compounds, and they are synthesized from the structure of echistatin and barbourin, respectively. In this review, we discuss some of the main functional and structural characteristics of this class of proteins and their potential for therapeutic use.

Keywords:
disintegrins
SVMP
ADAM
snake venom
integrins
RGD domain

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https://doi.org/10.1590/1678-9199-JVATITD-2023-0039
Received: 17 June 2023; Accepted: 25 August 2023; Published online: 18 September 2023

Background

Snake venom is a secretion produced in the glands located on both sides of the animal's upper jaw. Its evolutionary function includes the defense and survival of the snake, as well as the immobilization and digestion of prey, aiding in its feeding. It is a complex cocktail, as its composition is formed by the mixture of various compounds, predominantly proteins, peptides, amino acids, nucleic acids, carbohydrates, lipids, and metals [1, 2]. After its production in pairs of homologous glands, venom is secreted into the base of the fangs, which can be located in the posterior region (opisthoglyphous) or anterior region of the animal's mouth, with the latter case having either short and fixed fangs (proteroglyphous) or long and movable fangs (solenoglyphous) [2, 3].

Snakebite envenomation is considered a Neglected Tropical Disease with high incidence and severity, mainly affecting poverty regions [4]. It is estimated that around 5.4 million snakebites occur worldwide each year, resulting in 1.8 to 2.7 million cases of envenomation and approximately 81,000 to 138,000 deaths [5]. Snake venom exhibits a highly complex composition, and due to the diverse toxins with a wide range of biological functions, various clinical manifestations resulting from envenomation are observed, including local and systemic effects [6]. However, beyond its toxic action, snake venom is also recognized for its high therapeutic potential, as its composition contains approximately 100 to 500 pharmacologically active compounds capable of acting on different target sites. For this reason, many studies have been conducted in the search for alternative therapies for various diseases [7].

In this context, snake venomics has demonstrated great relevance for the more detailed analysis of venom components [8]. By using this strategy, which combines advances in proteomics and transcriptomics, it is possible to isolate venom compounds, estimate the content of toxins, as well as understand their biological and toxicological aspects [9]. Advances in these techniques have allowed the characterization of up to 20 families of proteins in the venom of a single snake, with some of these families containing up to 80 different toxins [10]. Despite the fascinating variability of compounds, most snake venoms are composed of four dominant protein families: phospholipase A_2 (PLA_2), three-finger toxins (3FTx), snake venom serine protease (SVSP), and snake venom metalloprotease (SVMP), along with secondary protein families, such as cysteine-rich secretory protein (CRISP), Kunitz peptides, L-amino acid oxidase (LAAO), natriuretic peptides, C-type lectins (CTL), disintegrins, among others [11].

In this review, we present the functional and structural aspects of disintegrins found in snake venom, as well as the evolutionary history of their emergence. We also discuss the potential applications of this class of peptides and the drugs already approved for therapeutic use.

What are snake venom disintegrins?

Snake venom disintegrins comprise a family of highly homologous, non-enzymatic polypeptides rich in cysteine (Cys). Their presence is described in the venom of snakes from the families Viperidae, Crotalidae, Atractaspididae, Elapidae, and Colubridae [12]. This family of small proteins interacts specifically with integrins, a group of cell adhesion receptors on the surface of certain cells, including platelets, vascular endothelial cells, and some tumor cells [13, 14]. This way, disintegrins, by preventing such binding, interfere in intercellular and cell-matrix interactions, as well as signal transduction [12, 14].

Integrins: a family of heterodimeric receptors

Integrins are transmembrane receptors that regulate or trigger different cellular processes upon binding to specific extracellular ligands [15]. They are heterodimeric proteins formed by the non-covalent association of α and β chains. In vertebrates, at least 18 α subunits and 8 β subunits have been identified, which can form a total of 24 different heterodimers. The α and β subunits of integrins do not have detectable homology between them, but there are conserved regions among different α subunits (approximately 30% identity) and among β subunits (around 45%) [16].

Integrins can recognize ligands from the extracellular matrix, cell surfaces, and other soluble ligands, with the αβ pairings of integrin subunits being determinants for binding specificity [16, 17]. Structurally, each integrin subunit consists of an extended multidomain extracellular region (up to 1104 residues in the α subunit and 778 residues in the β subunit), a transmembrane helix, and a short cytoplasmic tail (with 20 to 70 amino acids). The N-terminal portions of each subunit, located in the extracellular region, combine to form a globular ligand-binding "head" (Figure 1) [18, 19].

Integrins are present on the surface of many cell types and enable cell-cell interactions and interactions between cells and extracellular matrix proteins, including fibronectin, collagen, and laminin-1 [20]. These interactions are related to a wide range of biological effects, so the role of integrins is associated with physiological events such as cell adhesion [21], wound healing [22], regulation of neuronal connectivity [23], and synapses [24], as well as pathological effects as inflammation [17], tissue fibrosis [25], atherosclerotic plaque development [26], They also interfere in various stages of cancer development and progression, including survival, proliferation, angiogenesis, migration, invasion, survival in circulation, extravasation, and metastatic growth [12, 15, 17, 27–31].

Snake venom disintegrins: evolution from metalloproteases

Snake venom disintegrins are peptides derived from the proteolytic processing of snake venom metalloproteinase (SVMP) precursors and carry in their structure the recognition motifs for integrins RGD, KGD, WGD, VGD, MGD, RTS, KTS [13, 32]. SVMPs are found in large quantities in snake venom and are the main components responsible for the hemorrhagic action after snakebite, interfering with the victim's hemostatic system [33, 34]. They are divided into different subclasses based on size and domain structure. Class P-I SVMPs contain only the typical

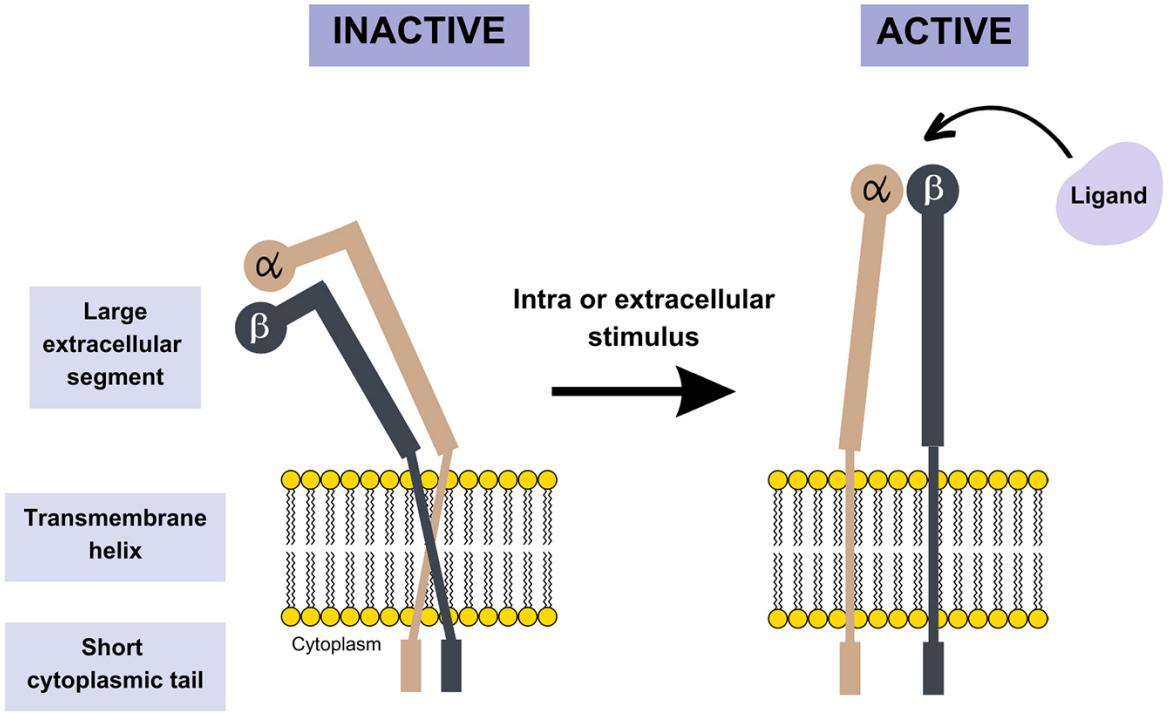

Figure 1. Integrin structure. Conversion of integrin from its inactive low-affinity conformation to the active high-affinity conformation for the ligand through intra- or extracellular stimuli.

metalloproteinase domain (M), composed of the pro-domain and proteolytic domain, and have a molecular mass of 20 to 30 kDa. Class P-II SVMPs have a molecular mass of 30 to 60 kDa and are structurally composed of pro-domain, proteolytic domain, and disintegrin-like domain (DI). Class P-III SVMPs (hemorrhagins) have a molecular mass between 60 to 100 kDa and are composed of a pro-domain, proteolytic domain, a disintegrin-like domain, and a cysteine-rich domain (C). In general, the hemorrhagic activity of these toxins depends on the M domain, but the DI and C domains are also important for their biological function. Thus, class P-III is recognized for its ability to induce higher and more diverse hemorrhagic activity when compared to class P-I and P-II SVMPs [33, 35, 36].

Evidence from molecular phylogenetics suggests that SVMPs evolved from a gene that encodes an A Disintegrin And Metalloprotease (ADAM) molecule, likely from an ancestral ADAM 7 or ADAM 28, belonging to the adamalysin family. Evolutionarily, SVMPs were recruited to the snake venom gland at the base of the advanced snake radiation, after the divergence of Pareatidae from the remaining Caenophidians, during the Paleogene period of the Cenozoic Era. The evolutionary history of SVMPs shows the loss of the cysteine-rich domain in class P-III, forming the SVMPs-PII, followed by the loss of the disintegrin-like domain and the formation of class P-I [35, 37].

Regarding domain organization and sequence, important similarities are observed between ADAMs and P-III SVMPs, including the presence of the pro-domain, proteolytic domain, disintegrin-like domain, and cysteine-rich domain. Regarding structural differences, ADAMs have an EGF domain, a transmembrane domain, and a cytoplasmic tail, which are not present in SVMPs [38].

The evolutionary history of disintegrins occurred through positive Darwinian selection, and their presence in snake venom results from the proteolytic processing of P-II metalloproteinases or translation of short messenger RNAs without the metalloproteinase coding region [39–42]. Thus, the presence of both free metalloproteinases and disintegrins can be observed in the venom [43].

Discovery and distribution of snake venom disintegrins

Snake venom disintegrins emerged in the scientific community in 1987, when Stefan Niewiarowski and Tur-Fu Huang isolated a low molecular weight non-enzymatic protein from the venom of *Trimeresurus gramineus*. The researchers observed that the protein, called trigramin, could block the binding of fibrinogen to stimulated GPIIb/IIIa receptors on platelets, thus inhibiting platelet aggregation. Although introduced in Toxinology in 1987, the term "disintegrin" was first used in 1990 when it was described as a new class of peptides isolated from snake venom, rich in the amino acid cysteine and containing an RGD domain in their structure [44, 45]. Since then, numerous studies have been conducted searching for this class of compounds in snake venom (Table 1). Approximately ten years after its discovery, non-RGD disintegrins were identified, challenging the concept of the obligatory presence of the Arg-Gly-Asp amino acids, and

paving the way for the future discovery of different integrin recognition motifs [46, 47].

Initially, disintegrins were studied for their inhibition of platelet aggregation due to the ability to interact with the transmembrane GPIIb/IIIa receptors (or αIIbβ3 integrin) present on the surface of platelets [39, 48–50]. Fibrinogen is a bivalent molecule capable of simultaneously binding to the activated GPIIb/IIIa receptor on two different platelets, forming bridges between the activated platelets [51–54]. Thus, disintegrins inhibit platelet aggregation by preventing the interaction of the αIIbβ3 integrin with fibrinogen.

Subsequently, in addition to their action on platelet receptors, many disintegrins have been isolated and characterized for their effects on other cells, demonstrating various biological functions, including interference with human neutrophil chemotaxis to sites of inflammation and tissue injury [55], antiparasitic activity [56], antiviral activity [57] and antitumor action through induction of apoptosis [50] and cytotoxicity [58], as well as inhibition of important steps in tumor development and progression, like adhesion [46, 59–63], angiogenesis [59, 64–67], migration [59, 62, 63, 68, 69] and metastasis [69–72].

Structural characterization of snake venom disintegrins

Snake venom disintegrins can be structurally classified into two major groups: monomeric and dimeric (Figure 2). Monomeric disintegrins are composed of three classes [73]. The first class consists of short disintegrins with 41 to 51 amino acid residues and four disulfide bonds. The second class comprises medium disintegrins with approximately 70 amino acids and six disulfide bonds. The third class of monomeric disintegrins contains long disintegrins with about 84 residues and seven disulfide bridges [74]. The second group of disintegrins is the dimeric disintegrins, which are further classified as homo- or heterodimers when the subunits are identical or different, respectively [73]. The subunits of dimeric disintegrins are composed of around 67 residues with ten cysteines, which are involved in forming four intrachain and two interchain disulfide bonds [74].

These proteins are highly homologous, and this structural similarity is primarily associated with the alignment of cysteine residues [75]. Figure 3 shows the analysis of multiple sequence alignments of disintegrin domains from five different structural classes, including Echistatin [76], Obtustatin [77], Barbourin [78], Tzabcanin [79], Cotiarin [80], Batroxostatin [81], Jarastatin [82, 83], Jararacin [82–84], Bitistatin [85], Salmosin-3 [86], Schistatin [87], Contortrostatin [48], CC5 [88], CC8 [88], EC3 [46] and EMF10 [47], highlighting conserved cysteine residues (Figure 3).

Regarding binding specificity, the correct pairing of cysteine residues is essential for exposing the motif that mediates the interaction with integrins and determining their inhibition [74]. In this context, the family of snake venom disintegrins can be divided into seven groups, each with a specific pattern

Table 1. Snake venom disintegrins isolation.

Disintegrin	Snake venom species	Motif	Publication data	Ref.
Trigramin	*Trimeresurus gramineus*	RGD	November-87	[44]
Echistatin	*Echis carinatus*	RGD	December-88	[76]
Applaggin	*Agkistrodon piscivorus piscivorus*	RGD	October-89	[110]
Albolabrin	*Trimeserusus albolabris*	RGD	May-90	[111]
Elegantin	*Trimeserusus elegans*	RGD	May-90	[111]
Flavoridin	*Trimeserusus flavoviridis*	RGD	July-90	[112]
Batroxostatin	*Bothrops atrox*	RGD	September-90	[81]
Eristostatin	*Eristicophis macmahoni*	RGD	November-90	[45]
Rhodostomin	*Calloselasma rhodostoma*	RGD	November-90	[45]
Triflavin	*Protobothrops flavoviridis*	RGD	February-91	[113]
Barbourin	*Sistrurus miliarius barbouri*	KGD	May-91	[78]
Basilicin	*Crotalus basilicus*	RGD	January-93	[84]
Cerastin	*Cerastes cereastes*	RGD	January-93	[84]
Cereberin	*Crotalus viridis cereberus*	RGD	January-93	[84]
Crotatoxin	*Crotalus atrox*	RGD	January-93	[84]
Cotiarin	*Bothrops cotiara*	RGD	January-93	[84]
Durissin	*Crotalus durissus durissus*	RGD	January-93	[84]
Jararacin	*Bothrops jararaca*	RGD	January-93	[84]
Lachesin	*Lachesis mutus*	RGD	January-93	[84]
Lutosin	*Crotalus viridis lutosus*	RGD	January-93	[84]
Molossin	*Crotalus molossus molossus*	RGD	January-93	[84]

Table 1. Cont.

Disintegrin	Snake venom species	Motif	Publication data	Ref.
Viridin	*Crotalus viridis viridis*	RGD	January-93	[84]
Contortrostatin	*Agkistrodon contortrix contortrix*	RGD	January-94	[114]
Multisquamatin	*Echis multisquamatus*	RGD	January-94	[114]
Flavostatin	*Trimeserusus flavoviridis*	RGD	May-96	[49]
Bitistatin	*Bitis arietans*	RGD	October-97	[115]
Salmosin	Agkistrodon *Halys Brevicaudus*	RGD	July-98	[116]
Accutin	*Agkistrodon acutus*	RGD	November-98	[64]
EC3	*Echis carinatus*	VGD/MLD	April-99	[46]
Rhodocetin	*Calloselasma rhodostoma*	?	May-99	[117]
Jarastatin	*Bothrops jararaca*	RGD	September-99	[82]
EMF-10	*Eristicophis macmahoni*	RGD/MGD	September-99	[47]
EC6	*Echis carinatus*	MLD/RGD	October-00	[118]
Alternagin-C	*Bothrops alternatus*	ECD	December-00	[119]
Lebein	*Macrovipera lebetina*	RGD	May-01	[120]
Trimestatin	*Trimeresurus flavoviridis*	RGD	September-01	[121]
Piscivostatin	*Agkistrodon piscivorus piscivorus*	RGD/KGD	September-01	[121]
Saxatillin	*Gloydius saxatilis*	RGD	January-02	[66]
CC5	*Cerastes cereastes*	RGD	January-02	[88]
CC8	*Cerastes cereastes*	RGD/WRG	January-02	[88]
Ocellatusin	*Echis ocellatus*	RGD	February-02	[122]
Bothrasperin	*Bothrops asper*	RGD	March-03	[123]
Obtustatin	*Macrovipera lebetina*	KTS	May-03	[77]
EO4	*Echis ocellatus*		June-03	[124]
EO5	*Echis ocellatus*	MLD/VGD	June-03	[124]
VA6	*Vipera ammodytes*	RGD	June-03	[124]
VB7	*Vipera berus*	RGD/KGD	June-03	[124]
VLO4	*Vipera lebetina obtusa*		June-03	[124]
VLO5	*Vipera lebetina obtusa*	VGD/MLD	June-03	[124]
Adinbitor	*Agkistrodon halys brevicaudus stejneger*	RGD	June-04	[125]
Viperistatin	*Vipera palestinae*	KTS	November-04	[126]
Bothrostatin	*Bothrops jararaca*	RGD	April-05	[127]
Jerdostatin	*Trimeresurus jerdonii*	RTS	December-05	[128]
Lebestatin	*Macrovipera lebetina*	KTS	December-05	[59]
Mojastin-1 and -2	*Crotalus scutulatus scutulatus*	RGD	April-06	[129]
DisBa-01	*Bothrops alternatus*	RGD	October-07	[128]
Viplebedin-2	*Vipera lebetina*	VGD/MLD	July-09	[113]
Disintegrin protein	*Naja naja*	?	August-12	[130]
Disintegrin	*Atropoides mexicanus*	RGD	December-14	[61]
Sasaimin	*Cerrophidion sasai*	RGD	December-14	[61]
Simusmin	*Crotalus simus*	RGD	December-14	[61]
Tzabcanin	*Crotalus simus tzabcan*	RGD	September-15	[79]
Disintegrin_CC	*Cerastes cereastes*	RGD	December-17	[131]
Disintegrin	*Crotalus durissus collilineatus*	Non-RGD	October-18	[132]
Cerastategrin	*Cerastes cereastes*	RGD	September-20	[133]

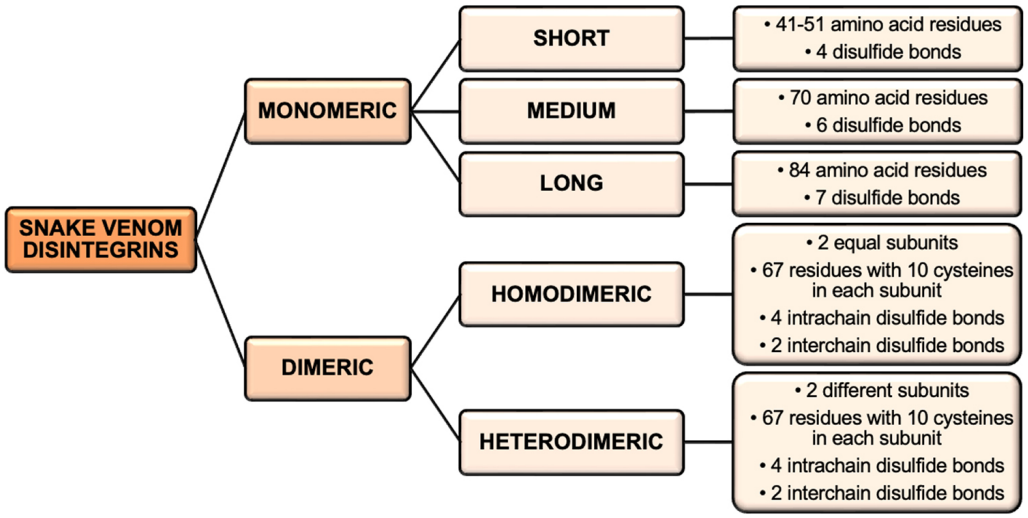

Figure 2. Structural classification of disintegrins.

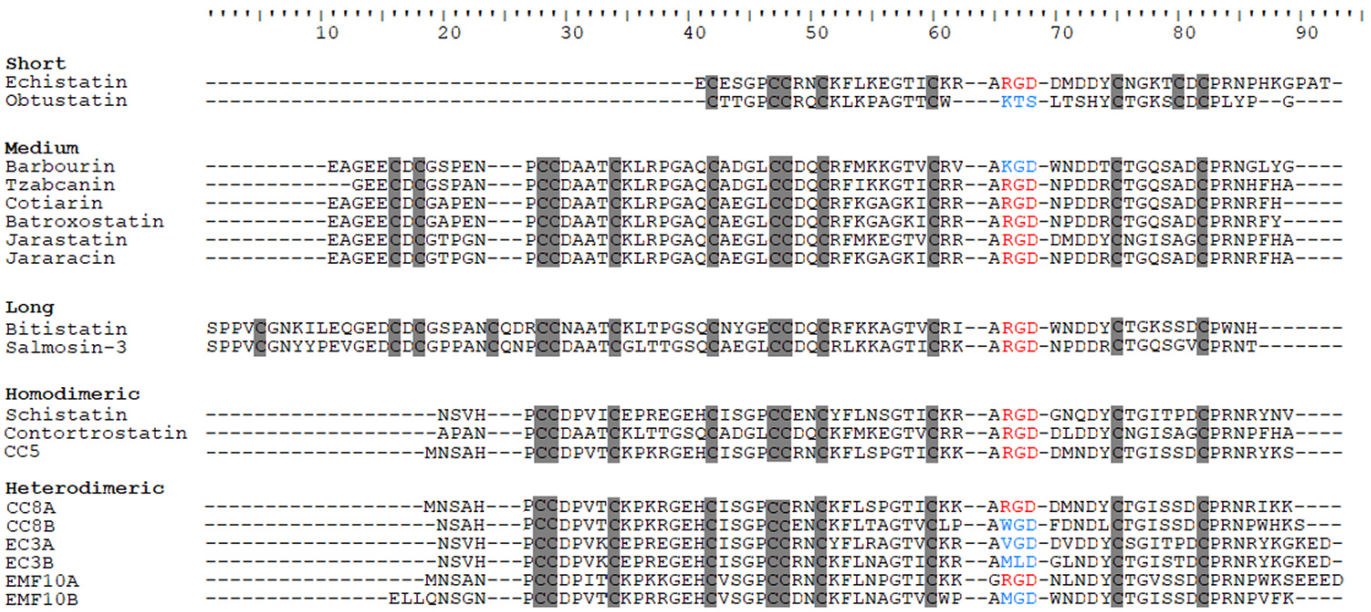

Figure 3. Multiple alignments among selected disintegrins from different structural classes. Cysteine residues are highlighted in gray. The integrin-binding RGD motif is represented in red, and non-RGD motifs are in blue.

of sequence and disulfide bond formation between cysteine residues (Figure 4). Group 1 includes the disintegrin-like domain of proteins from the ADAM/SVMP subfamily. Its disulfide pattern is defined as Cys1-Cys5, Cys2-Cys3, Cys4-Cys10, Cys7-Cys9, Cys8-Cys13, Cys11-Cys14, while Cys6 and Cys12 form connections with other domains of the protein. Group 2 consists of disintegrins similar to Bitistatin A, and Cys1-Cys4, Cys2-Cys7, Cys3-Cys6, Cys5-Cys11, Cys8-Cys10, Cys9-Cys13, Cys12-Cys14 characterize their disulfide pattern. Group 3 is formed by disintegrins similar to Bitistatin B, and their disulfide bond pattern consists of Cys1-Cys7, Cys2-Cys6, Cys3-Cys4, Cys5-Cys11, Cys8-Cys10, Cys9-Cys13, Cys12-Cys14. Group 4 consists of monomeric disintegrins similar to Kistrin, and the disulfide pattern of these molecules is Cys1-Cys5, Cys2-Cys4, Cys3-Cys9, Cys6-Cys8, Cys7-Cys11, Cys10-Cys12. Group 5 is the Salmosin group, also composed of monomeric disintegrins, and their disulfide pattern is Cys1-Cys3, Cys2-Cys4, Cys5-Cys8, Cys7-Cys9, Cys6-Cys11, Cys10-Cys12. Group 6 includes dimeric disintegrins, with an intrachain disulfide pattern characterized by Cys1-Cys7, Cys4-Cys6, Cys5-Cys9, Cys8-Cys10, while Cys2 and Cys3 form a disulfide bridge with the other subunit of the dimer. Lastly, group 7 comprises short disintegrins, and the disulfide pattern of these molecules can be described as Cys1-Cys4, Cys2-Cys7, Cys3-Cys6, and Cys5-Cys8 [89].

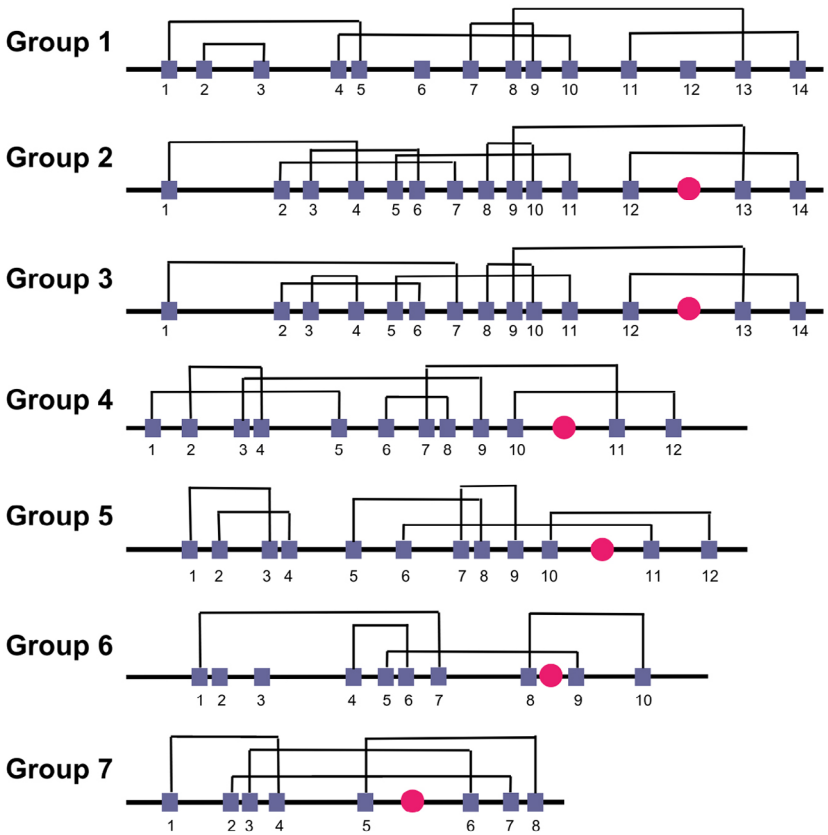

Figure 4. Disulfide bonding pattern for each group within the disintegrin family. (**Group 1:**) DAM/SVMP subfamily-like disintegrin domain proteins; (**Group 2:**) Bitistatin A-like disintegrins; (**Group 3:**) Bitistatin B-like disintegrins; (**Group 4:**) Kistrin-like disintegrins; (**Group 5:**) Salmosin-like disintegrins; (**Group 6:**) Dimeric disintegrins; (**Group 7:**) Short disintegrins. Purple squares indicate cysteine residues, while pink circle indicates the integrin-binding motif.

Function and potential applications of snake venom disintegrins

Snake venom disintegrins can selectively bind to integrins, which are strongly tied to the specific motifs found in their structure [90] (Figure 5). This way, during envenomation, they exhibit a wide array of functions, serving various crucial roles, like binds to platelet receptors, impeding their aggregation, and resulting in the onset of bleeding disorders [91]. Consequently, disintegrins contribute to disrupting hemostatic processes (Table 2).

Some snake venom disintegrins can inhibit bone resorption *in vitro* [92] and can also be used as a diagnostic tool. An example, we cite bitistatin, which can potentially be used in molecular imaging of thromboembolic diseases [93].

It has also been demonstrated that disintegrins can interfere with the chemotaxis of human neutrophils to sites of inflammation and tissue injury [55] and exhibit antiparasitic activity against *Leishmania infantum* promastigotes [56].

Intriguingly, certain disintegrins have demonstrated notable anti-tumor and anti-angiogenic properties (Table 3). This remarkable feature opens up new possibilities for their utilization as potential therapeutic agents in cancer treatment, and by targeting tumor growth and impeding blood vessel formation, these disintegrins exhibit promising potential in medical research and innovation.

Snake venom disintegrins: from lab bench to market

Animal venoms are rich mixtures of components that may have important pharmacological actions. Many of these components have already been extensively studied to become drugs, and after approval by the Food and Drug Administration (FDA), turned into widely used molecules [94].

A very important example of a drug derived from animal toxins is captopril (Capoten®, Bristol-Myers Squibb, New York, NY, EUA), which is widely used against hypertension [95]. This was the first animal-derived drug approved by the FDA in 1981, which mechanism is responsible for inhibiting the angiotensin-converting enzyme (ACE). Thus, the production of angiotensin II is also inhibited, reducing hypertension effects, and increasing the hypotensive action of bradykinin, known as a bradykinin potentiating factor (BPF) [96–99]. Although it is a very effective natural molecule, the captopril used in medicaments is a synthetic molecule based on the miniaturization of the original molecule and chemically modified to be administered orally [94, 100]. In sequence, in 1985, the FDA approved Enalapril (Vasotec®, Merck, Darmstadt, Germany), which was also used to treat hypertension and congestive heart failure [94, 101].

Some disintegrins have been extensively studied and are nowadays FDA-approved drugs as well. Tirofiban (Aggrastat®,

Table 2. Snake venom disintegrins that can act on the hemostatic system.

Disintegrin (snake venom)	Motif	Integrins	Action	Ref.
Accutin (*Agkistrodon acutus*)	RGD	αIIbβ3	Inhibit human platelet aggregation induced by ADP, collagen, fibrinogen, thrombin and the thromboxane analogue U46619 Inhibit platelet aggregation of platelet-rich plasma	[134]
Albolabrin (*Trimeserusus albolabris*)	RGD	αIIbβ3	Block platelet-fibrinogen interaction Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[111,135]
Applagin (*Agkistrodon piscivorus piscivorus*)	RGD	αIIbβ3	Block platelet aggregation induced by ADP, collagen, thrombin, and arachidonic acid	[110]
Barbourin (*Sistrurus miliarius barbouri*)	KGD	αIIbβ3	Inhibit fibrinogen to bind αIIbβ3 integrin	[78]
Basilicin (*Crotalus basilicus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Bitistatin (*Bitis arietans*)	RGD	αIIbβ3	Block platelet-fibrinogen interaction Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[135]
CC5 (*Cerastes cereastes*)	RGD	αIIbβ3	Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[88]
CC8 (*Cerastes cereastes*)	RGD/ WRG	αIIbβ3	Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[88]
Cerastin (*Cerastes cereastes*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Cereberin (*Crotalus viridis cereberus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Contortrostatin (*Agkistrodon contortrix contortrix*)	RGD	αIIbβ3	Inhibit ADP-induced platelet aggregation of platelet-rich plasma from humans, dogs and rabbits	[114]
Crotatoxin (*Crotalus atrox*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Cotiarin (*Bothrops cotiara*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Durissin (*Crotalus durissus durissus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
EC3 (*Echis carinatus*)	VGD/ MLD	αIIbβ3	Inhibit fibrinogen to bind αIIbβ3 integrin	[137]
Echistatin (*Echis carinatus*)	RGD	αIIbβ3	Block platelet-fibrinogen interaction Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[135]
Elegantin (*Trimeserusus elegans*)	RGD	αIIbβ3	Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[111]
EMF-10 (*Eristicophis macmahoni*)	RGD/ MGD	αIIbβ3	Inhibit ADP-induced platelet aggregation	[47]
Eristostatin (*Eristicophis macmahoni*)	RGD	αIIbβ3	Able to bind in ADP-, thrombin-induced, and resting platelet	[138]
Flavoridin (*Trimeserusus flavoviridis*)	RGD	αIIbβ3	Block platelet-fibrinogen interaction Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[135]
Jararacin (*Bothrops jararaca*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit ADP- and thrombin-induced platelet aggregation Inhibit adhesion to vitronectin, and fibrinogen to binding integrins	[136,139]
Jarastatin (*Bothrops jararaca*)	RGD	αIIbβ3	Inhibit ADP- and thrombin-induced platelet aggregation	[139]
Jerdostatin (*Trimeresurus jerdonii*)	RTS	αIIbβ3	Inhibit fibrinogen to bind αIIbβ3 integrin	[140]
Lachesin (*Lachesis mutus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]

Table 2. Cont.

Disintegrin (snake venom)	Motif	Integrins	Action	Ref.
Lebein (*Macrovipera lebetina*)	RGD	?	Inhibit ADP-induced platelet aggregation of platelet-rich plasma	[120]
Lutosin (*Crotalus viridis lutosus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Mojastin-1 and -2 (*Crotalus scutulatus scutulatus*)	RGD	α5β1	Inhibit ADP-induced platelet aggregation of whole blood	[129]
Molossin (*Crotalus molossus molossus*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]
Multisquamatin (*Echis multisquamatus*)	RGD	αIIbβ3	Inhibit ADP-induced platelet aggregation of platelet-rich plasma from humans, dogs and rabbits	[114]
Rhodocetin (*Calloselasma rhodostoma*)	?	?	Inhibit collagen-induced platelet aggregation	[117]
Saxatillin (*Gloydius saxatilis*)	RGD	αIIbβ3	Inhibit the interaction of integrins and fibrinogen Inhibit ADP-induced platelet aggregation	[66]
Triflavin (*Protobothrops flavoviridis*)	RGD	αIIbβ3	Inhibit ADP-induced and resting platelet	[113]
Trigramin (*Trimeresurus gramineus*)	RGD	αIIbβ3	Inhibit the interaction of ADP-induced platelet and fibrinogen Inhibit chymotrypsin-treated platelet aggregation Bind to resting platelet	[44]
Viplebedin-2 (*Vipera lebetina*)	VGD/ MLD	?	Inhibit ADP- and collagen-induced platelet aggregation Inhibit platelet adhesion	[137]
Viridin (*Crotalus viridis viridis*)	RGD	αvβ3 α5β1 αIIbβ3	Inhibit platelet aggregation, adhesion to vitronectin, and fibrinogen to binding integrins	[136]

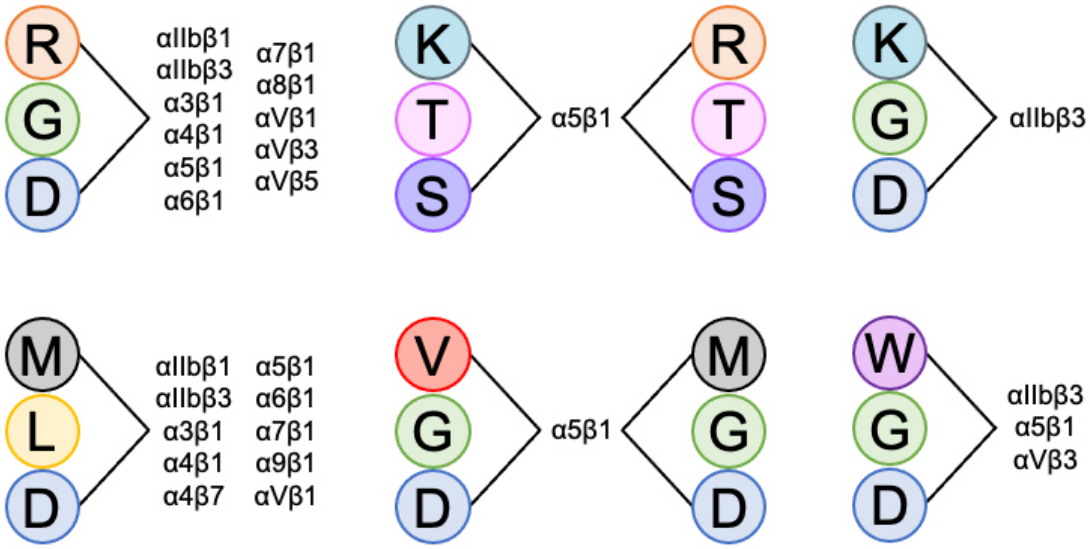

Figure 5. Interaction of snake venom disintegrins motifs with different integrins.

Medicure International, Inc., Winnipeg, Manitoba, Canada) is also a synthetic drug based on the RGD domain of echistatin from *Echis carinatus* [102]. Furthermore, it has a chemical modification that increases its interaction with platelet glycoproteins, specifically with their GPIIb/IIIa receptors [76]. Thus, this drug can inhibit platelet aggregation and other thrombotic actions due to its competition with fibrinogen for the recognition site of the RGD domain in the GPIIb/IIIa receptor [102, 103]. Tirofiban was approved by the FDA in 1998 as a treatment for acute coronary syndrome [104].

Table 3. Discovery of snake venom disintegrins that can act as anticancer agents.

Disintegrin (snake venom)	Motif	Cell line (cancer type)	Integrins	Action	Ref.
Accutin (*Agkistrodon acutus*)	RGD	HUVEC (human non-cancer cell)	αvβ3	Induce apoptosis Inhibit angiogenesis *in vitro* and *in vivo*	[141]
Albolabrin (*Trimeserusus albolabris*)	RGD	B16-F10 (murine melanoma)	α5β1 αvβ3 α6β1	Inhibit cell-matrix attachment in vitro Inhibit metastasis of tumor cells	[142]
Alternagin-C (*Bothrops alternatus*)	ECD	HUVEC (human non-cancer cell) MDA-MB-231 (human breast cancer) HMEC-1 (human cells from tumor microenvironment) Human fibroblasts	α2β1	Modulates cell adhesion, migration and proliferation Inhibit adhesion, viability and migration of VEGF-induced cell Inhibit angiogenesis *in vitro* Infer in tumor progression	[143–145]
Barbourin (*Sistrurus miliarius barbouri*)	KGD	B16-F10 (murine melanoma)	αvβ3 αvβ1	Inhibit cell adhesion	[146]
Bitistatin (*Bitis arietans*)	RGD	HUVEC (human non-cancer cell)	αvβ3	Inhibit cell adhesion	[147]
CC5 (*Cerastes cereastes*)	RGD	A5 (murine non-cancer cell) JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) CHO K1 (murine non-cancer cell)	α5β1 αvβ3	Inhibit cell adhesion	[88]
CC8 (*Cerastes cereastes*)	RGD/ WRG	A5 (murine non-cancer cell) JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) CHO K1 (murine non-cancer cell)	α5β1 αvβ3	Inhibit cell adhesion	[88]
Contortrostatin (*Agkistrodon contortrix contortrix*)	RGD	M24 met (human metastatic melanoma)	α5β1 αvβ1	Inhibit cell adhesion *in vitro* Inhibit lung colonization *in vivo*	[148]
DisBa-01 (*Bothrops alternatus*)	RGD	HMEC-1 (human non-cancer cell) MDA-MB-231 (human breast cancer) B16-F10 (murine melanoma)	αvβ3	Inhibit angiogenesis Inhibit cell adhesion and proliferation	[149]
Disintegrin (*Crotalus durissus collilineatus*)	Non-RGD	MDA-MB-231 (human breast cancer)	?	Inhibit cell migration	[132]
EC3 (*Echis carinatus*)	VGD/ MLD	A5 (murine non-cancer cell) VNRC3 (murine non-cancer cell) CHO (murine non-cancer cell) JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia) CHO K1 (murine non-cancer cell) RPMI886 (human chronic myelogenous leukaemia)	αIIbβ3 α5β1 αvβ3 α4β1 α4β7	Inhibit cell adhesion	[46]
EC6 (*Echis carinatus*)	MLD/ RGD	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α5β1 α4β1	Inhibit cell adhesion	[118]
Echistatin (*Echis carinatus*)	RGD	A5 (murine non-cancer cell) JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) SW480 (human colon adenocarcinoma) Jurkat (human acute T cell leukemia)	α5β1 αvβ3	Inhibit cell adhesion Inhibit angiogenesis	[150]
EMF-10 (*Eristicophis macmahoni*)	RGD/ MGD	K562 (human myelogenous leukemia)	α5β1	Inhibit cell adhesion	[47]

Table 3. Cont.

Disintegrin (snake venom)	Motif	Cell line (cancer type)	Integrins	Action	Ref.
EO5 (*Echis ocellatus*)	MLD/ VGD	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α4β1	Blocked cell adhesion	[124]
Eristostatin (*Eristicophis macmahoni*)	RGD	A375 (human malignant melanoma) HT1080 (human fibrosarcoma)	αIIbβ3 α5β1 αvβ3	Inhibit cell adhesion	[151]
Jerdostatin (*Trimeresurus jerdonii*)	RTS	JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) SW480 (human colon adenocarcinoma) Jurkat (human acute T cell leukemia)	αIIbβ3 α5β1 α1β1 α2β1 α6β1 αvβ3 α4β1 α9β1	Inhibit cell adhesion	[140]
Lebein (*Macrovipera lebetina*)	RGD	LS174, HCT116, and HT29 (human colon adenocarcinoma) SK-MEL-28 and LU-1205 (human melanoma)	α5β1 αvβ3	Induce apoptosis Inhibit cell migration and adhesion Inhibit angiogenesis by down-regulating VEGF and NRP1 Expression	[152,153]
Lebestatin (*Macrovipera lebetina*)	KTS	CHO (murine non-cancer cell) HT29-D4 (human colonic adenocarcinoma) HT1080 (human fibrosarcoma) K562 (human myelogenous leukemia) IGROV1 (human ovarian adenocarcinoma) HMEC-1 (human non-cancer cell) PC12 (rat pheochromocytoma)	α1β1	Inhibit cell migration and adhesion Inhibit angiogenesis	[59]
Mojastin-1 and -2 (*Crotalus scutulatus scutulatus*)	RGD	BXPC-3 (human pancreatic adenocarcinoma)	α3β1	Inhibit cell proliferation, migration and adhesion Induce apoptosis	[154]
Obtustatin (*Macrovipera lebetina*)	KTS	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α1β1	Inhibit angiogenesis *in vivo*	[77,150]
Purpureomaculin (*Trimeresurus purpureomaculatus*)	RGD	MCF-7 (human breast adenocarcinoma)	αvβ5	Inhibit cell growth	[155]
Rhodocetin (*Calloselasma rhodostoma*)	?	HT1080 (human fibrosarcoma)	α2β1	Inhibit cell adhesion and migration	[156]
Rhodostomin (*Calloselasma rhodostoma*)	RGD	B16-F10 (murine melanoma) HUVEC (human non-cancer cell)	αvβ3	Inhibit angiogenesis Suppress tumor growth *in vivo* Inhibit cell proliferation	[157]
Saxatillin (*Gloydius saxatilis*)	RGD	HUVEC and SMC (human non-cancer cells) MDAH2774 (human ovarian cancer cells)	αvβ3	Inhibit cell proliferation, migration and adhesion Inhibit angiogenesis Inhibit tumor metastasis	[66,158,159]
Triflavin (*Protobothrops flavoviridis*)	RGD	B16-F10 (murine melanoma)	αIIbβ3	Inhibit cell adhesion	[160]
Tzabcanin (*Crotalus simus tzabcan*)	RGD	A-357 (human malignant melanoma) Colo-205 (human colorectal adenocarcinoma) MCF-7 (human breast adenocarcinoma) A-549 (human lung adenocarcinoma)	αvβ3	Inhibit cell migration and adhesion	[79,161]

Table 3. Cont.

Disintegrin (snake venom)	Motif	Cell line (cancer type)	Integrins	Action	Ref.
VA6 (*Vipera ammodytes*)	RGD	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α5β1	Inhibit cell adhesion	[124]
VB7 (*Vipera berus*)	RGD/ KGD	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α5β1	Inhibit cell adhesion	[124]
Viperistatin (*Vipera palestinae*)	KTS	A5 (murine non-cancer cell) JY (human lymphoblastoid cell) K562 (human myelogenous leukemia) SW480 (human colon adenocarcinoma)	α1β1	Inhibit cell adhesion	[126]
VLO5 (*Vipera lebetina obtusa*)	VGD/ MLD	A5 (murine non-cancer cell) K562 (human myelogenous leukemia) Jurkat (human acute T cell leukemia)	α4β1	Block cell adhesion	[124]

Another antiplatelet compound, Eptifibatide (Integrilin®, Millennium Pharmaceuticals, Inc.), was also approved by the FDA in 1998, and licensed in 2005, to Schering-Plough [94]. Its development coincided with the research for the synthetic peptide analogs of barbourin, a disintegrin from *Sistrurus miliarius barbouri* [78]. The conservative substitution of arginine (R) amino acids with lysine (K) in barbourin enhances its specificity towards the platelet glycoprotein complex GPIIb/IIIa compared to other disintegrins containing the RGD motif [78]. However, this specificity may also be influenced by the size of the peptide ring formed by disulfide bridges and the amino acids near the KGD domain. As a result, new peptides have been synthesized for potential clinical use, such as Eptifibatide, a synthetic heptapeptide that is more resistant to proteolysis [105–107].

Since the approval of the first venom-derived drug and the beginning of disintegrins' saga in Toxinology [44], it took over 10 years of research and effort for the first medication derived from snake venom disintegrins also to be approved (Figure 6). However, it was already known that venoms and their components could cause modifications in the human body, and their applicability in clinical settings had been recognized.

Currently, a product based on snake venom toxins has been attracting attention: Heterologous Fibrin Sealant. This sealant is composed of a thrombin-like enzyme from *Crotalus durissus terrificus* venom and fibrinogen-rich cryoprecipitate extracted from the blood of *Bubalus bubalis buffaloes*. It can be used for the treatment of chronic venous ulcers, as demonstrated in phase I/II clinical trials, highlighting its effectiveness and safety [108]. While there are currently no clinical studies using snake venom disintegrins, human disintegrins, especially ADAMs, have been targeted for the therapy of other pathological conditions in clinical trials, such as cirrhosis and portal hypertension (NCT04267406), epithelial dysfunction (NCT00898859), idiopathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension (NCT05478226), among others [109].

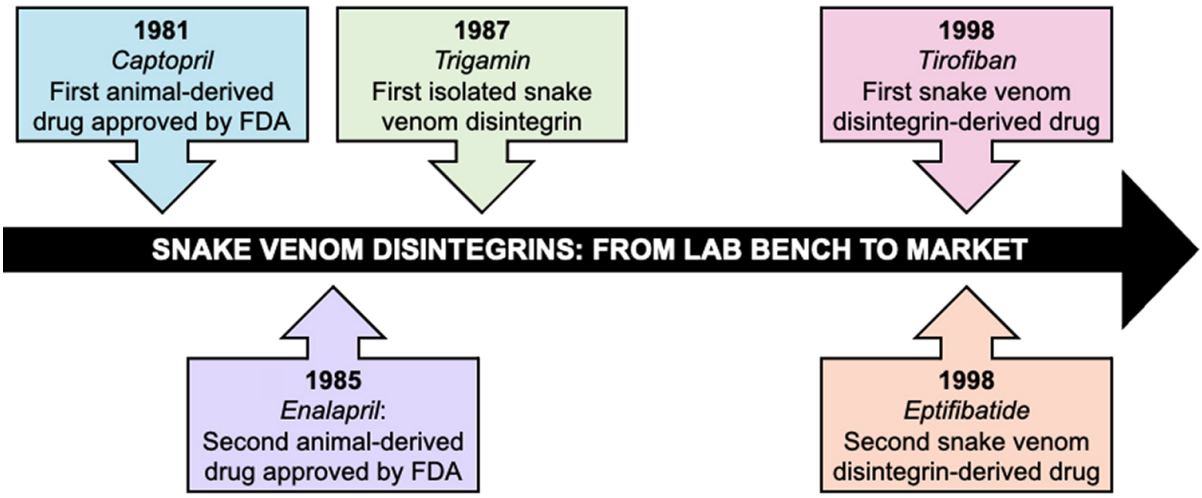

Figure 6. Timeline of snake venom disintegrins, from the beginning of disintegrins' saga in Toxinology until their FDA approval.

Conclusion

Snake venom disintegrins' saga was started in 1987 and classified these molecules as small peptides that can inhibit the function of integrins, which are cell surface receptors involved in various cellular processes like cell adhesion, migration, and signaling. Integrins are important for cell adhesion to extracellular matrix proteins, mediating cell-cell interactions, and interfering in integrin-mediated processes, as snake venom disintegrins can have various effects on cells and tissues.

Among their unique properties, snake venom disintegrins can inhibit platelet aggregation, *i.e.*, bind to integrins on platelets, preventing their aggregation and potentially disrupting the clotting process. Consequently, two important antiplatelet drugs were based on disintegrins from snake venoms, and they are on the market nowadays.

Moreover, snake venom disintegrins have shown anti-cancer properties by targeting integrins that are overexpressed in specific cancer cells and blocking integrin-mediated signaling pathways. These disintegrins can also inhibit tumor growth and metastasis. Notably, although snake venom disintegrins possess therapeutic potential, they exhibit high potency and can manifest toxicity. Thus, rigorous investigation is required before contemplating snake venom disintegrin use in medical applications.

Abbreviations

ACE: angiotensin-converting enzyme; ADAM: a disintegrin and metalloprotease; BPF: bradykinin potentiating factor; C: cysteine-rich domain; CRISP: cysteine-rich secretory protein; CTL: C-type lectins; DI: disintegrin-like domain; FDA: Food and Drug Administration; 3FTx: three-finger toxins; LAAO: L-amino acid oxidase; M: typical metalloproteinase domain; PLA_2: phospholipase A_2; SVMP: snake venom metalloproteases; SVSP: snake venom serine protease.

Availability of data and materials

Not applicable.

Funding

This study was supported by *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo Research Foundation, scholarships to GOA n. 2020/15670-5 and n. 2022/05554-3 and to ISO n. 2020/13176-3 and n. 2022/08964-8, grants to ECA n. 2019/10173-6 and n. 2021/11936-3 and to SVS n. 2022/04804-6), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Coordination of Superior Level Staff Improvement, scholarship to GOA n. 88887.661488/2022-00) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, The National Council for Scientific and Technological Development, scholarships to ECA n. 309399/2021-1 and SVS n. 303781/2022-0).

Competing interests

The authors declare that they have no competing interests.

Author contributions

GOA and ISO conceived the main idea of this work and drafted the manuscript. ECA provided essential contributions to the manuscript. SVS was a major contributor to writing the manuscript. SVS and ECA review the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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