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Scientific and Standardization Committee Communications: Inventory Of Exogenous Inhibitors of Platelet Aggregation From Animal Sources

On behalf of the Registry of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis

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Several exogenous factors isolated from animal sources affect platelet function and aggregation; some of them induce, whereas others inhibit platelet aggregation. This inventory deals with inhibitors from animal sources that have appeared in the literature until mid-1999.

Inhibitors of Platelet Aggregation

The initial inventory of platelet aggregation inhibitors (1) dealt with those isolated from snake venoms. This updated inventory includes in addition to several new inhibitors platelet aggregation from snake venoms, those that were isolated from other animal sources. They are proteins or glycoproteins with their molecular weight ranging from 5000 to several tens of thousands. These factors inhibit platelet aggregation by different mechanisms. A large number of these inhibitors do not exhibit any enzymatic activity. In contrast, some of them exhibit enzymatic activities, such as phospholipase A_2 (PLA_2), proteinase and nucleotidase. In general, the mechanism of inhibition of platelet aggregation is well understood for several groups of nonenzymatic proteins. However, further research is required to delineate the mechanism of inhibition by some of the enzymes.

Nonenzymatic inhibitors

Based on the mechanism of inhibition, we have classified the nonenzymatic factors into five classes.

Class I: *Antagonists of fibrinogen receptor glycoprotein IIb/IIIa complex (GPIIb/IIIa).* Interaction between fibrinogen and GPIIb/IIIa complex is the important final step to platelet aggregation. Since most platelet agonists go through this final step, these antagonists inhibit platelet aggregation with similar, if not identical, IC_{50} values irrespective of the agonist used to initiate aggregation. Several different classes of proteins have been shown to interfere at this critical step. Disintegrins are the most well studied aggregation platelet inhibitors. Although initial studies of purification and characterization of this group of proteins from snake venoms were done in the mid-1980's (2, 3), it was the simultaneous determination of the complete amino acid sequences of

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echistatin and trigramin (4, 5) that triggered immense research interest in both academia and pharmaceutical industries. These polypeptides contained the RGD tripeptide sequence, the short segment involved in adhesive reactions. Todate more than 40 disulfide-rich polypeptides, known as disintegrins because of their ability to interfere in the integrin activity, have been purified from snake venoms alone (Table 1; Figure 1). On a molar basis, these polypeptides are 1000 – 30,000 times more potent inhibitors of platelet aggregation than the linear RGDS peptide (6). They are most commonly found in crotalid and viperid snake venoms, but rarely found in elapid and hydrophid snake venoms. Disintegrins are classified into three distinct subfamilies based on their size (7) (Figure 1). Short chain disintegrins contain 47-51 amino acid residues and four disulfide bridges; medium chain disintegrins contain 68-75 amino acid residues and six disulfide bridges; and long chain disintegrins contain 83-84 amino acid residues and seven disulfide bridges. Based on the structural analysis of the sequences, we proposed that disintegrins are proteolytic products of longer precursor proteins (8, 9). Since then cDNA and amino acid sequences of several precursors have been determined (10-19). These results support the presence of common precursors for metalloproteinases, disintegrins and related proteins. The proteolytic processing to form various subfamilies of disntegrins is most likely be determined by intradomain disulfide bridges and the accessibility of interdomain regions, along with glycosylation (9).

Disintegrins bind to GPIIb/IIIa on both resting and activated platelets with high affinities (Kd, $10^{-7} \sim 10^{-8}$ M) in the presence of divalent cations. Irrespective of their length, they contain the RGD tripeptide sequence in a homologous position and it is described to be essential for their inhibitory properties. Only two disintegrins contain KGD in the homologous position (20, 21). The authors claim that this makes barbourin (20) a specific inhibitor of interaction between fibrinogen and GPIIa/IIIb and went on to develop several cyclic peptides with KGD sequence as potent inhibitors of platelet aggregation (22). Three-dimensional structures of four disintegrins have been determined by NMR techniques (23-27). The R/KGD segment is located on the tip of a hairpin loop that is stabilized by disulfide bridges. The residues in the immediate neighborhood of this RGD segment appear to contribute significantly to the inhibitory potency of disintegrins (28, 29).

As mentioned above, there are some venom proteins that contain disintegrin/disintegrin-like domain and these may inhibit platelet aggregation. In some cases, they may also contain metalloproteinase domains that may proteolytically alter the functions of the target protein (see below).

Based on the structure of disintegrins, a variety of cyclic R/KGD peptide or peptide-mimetic derivatives have been developed that are under clinical trials and some of them are found to be effective in reducing ischemic complication, particularly the restenosis after percutaneous transluminal coronary angioplasty (PTCA) (30, 31). In addition to their role in the development of antithrombotic agents and treatment of cardiovascular diseases, they may also be useful in the treatment of cancer growth and tumor metastasis as well as osteoporosis. Disintegrins have been shown to inhibit the adhesion between tumor cell and extracellular matrices (32-35). They also inhibit experimental metastasis of melanoma (36-39). Some of them inhibit tumor cell-induced platelet aggregation (40). Further, disintegrins through the blockade of $\alpha_v\beta_3$ integrin show potent antiangiogenic effects (41, 42). Disintegrins block the osteoclast-mediated bone resorption by

interfering in the $\alpha_v\beta_3$ integrin activity (43, 44) and prevent bone loss in mice and rats (45, 46). Thus the study of disintegrins contributes significantly to both academic and clinical research.

Apart from disintegrins, there are other inhibitors of platelet aggregation, which contain the RGD tripeptide in their structure. These proteins are isolated from snake venom, leeches and ticks (Table 2). These polypeptides do not share any significant structural homology with disintegrins (Figures 2 and 3). For example, mambin (or dendroaspin), a platelet aggregation inhibitor isolated from *Dendroaspis j. kaimosae* snake venom (47), shows a striking resemblance to the short-chain postsynaptic neurotoxins (48) (Figure 2). Although ornatins and decorsin were isolated from leaches, they do not have significant similarity except for RGD containing carboxy terminal segment (49, 50) (Figure 3). Variabilin from the salivary glands of hard tick *Dermacentor variabilis*, despite the presence of RGD, has little homology to any other GPIIb/IIIa antagonists (51).

Disagregin, a potent inhibitor of platelet aggregation isolated from the soft tick *Ornithodoros moubata* (52) is a GPIIb/IIIa antagonist. Surprisingly, it does not contain the tripeptide RGD (Figure 2). The structure-function relationship of this polypeptide will be of great interest.

Class II*: Antagonists of von Willebrand factor receptor glycoprotein Ib (GPIb).* Binding of plasma von Willebrand Factor (vWF) to the platelet glycoprotein, GPIb is assumed to play a key role in the earliest stages of platelet aggregation (53, 54). So far this group of inhibitors has been purified only from snake venoms (Table 3). They are heterodimeric with the two subunits (α and β subunits) linked through a disulfide bridge. Only flavocetin-B from *Trimeresurus flavoviridis* (55) has 3 subunits. All subunits are structurally similar to Ca^{2+}-dependent (C-type) lectins. These proteins prevent platelet aggregation by binding to the GPIb and thus blocking the interaction between vWF and its receptors. It has been shown that the GPIb binding site resides on the β-chain subunit (56). Unlike other inhibitors in this group, echicetin isolated from *Echis carinatus* also inhibits platelet aggregation induced by α-thrombin (56, 57).

Class III*: Antagonists of collagen-platelet interaction.* Subendothelial collagen serves as an anchorage for circulating platelets in the process of hemostasis and thrombosis (58). Platelets adhere to exposed subendothelial collagen through GPIa/IIa receptors, become activated and the activated platelets aggregate. Some of the platelet aggregation inhibitors interfere with the interaction of collagen with platelet receptors (Table 4). They have been isolated from snake venoms, leech, soft tick and triatome bug. The inhibitors from snake venoms, similar to disintegrins, are derived from large metalloproteinase/disintegrin precursor proteins. Catrocollastatin-C, atrolysin A/DC and jararhagin-C have the disintegrin-like and cysteine-rich domains (59-61). The former two proteins inhibit platelet aggregation; catrocollastatin-C specifically binds to collagen and inhibits only collagen-induced aggregation, whereas atrolysin A/DC also inhibits ADP-induced aggregation (59, 60). Their longer counterparts containing a metalloproteinase domain, namely catrocollastatin, atrolysin A and jararhagin, respectively, also inhibit collagen-induced platelet aggregation (60, 62, 63). Jararhagin, in contrast, binds to GPIa and cleaves GPIIa (64) (see below). Since jararhagin-C has identical sequence to the C-terminal region of jararhagin (61), and since catrocollastatin-C and atrolysin A/DC by themselves inhibit platelet aggregation, it is conceivable that jararhagin-C too acts as a collagen receptor antagonist. A similar situation may also be true in the case of crovidisin (65). The inhibitors from leech and soft tick bind specifically to collagen fibers and interfere in the interaction between collagen and its receptor GPIa/IIa (66-

71). Although pallidin specifically inhibits collagen-induced platelet aggregation similar to all the above proteins, the mechanism of inhibition by pallidin is not yet clear (72, 73).

***Class IV**: Antagonists of thrombin-thrombin receptor interaction.* Thrombin is a key enzyme in thrombosis and hemostasis. In addition to its main role in the formation of the fibrin clot in blood coagulation, it activates different types of cells, particularly platelets and acts as a potent inducer of platelet aggregation. Three functional sites have been recognized in thrombin; the active site, anion-binding exosite I, and anion-binding exosite II. Anion-binding exosite I mediates thrombin binding to fibrinogen, the platelet receptor and thrombomodulin, whereas the anion-binding exosite II is the heparin-binding site (74). Some thrombin inhibitors are shown to be inhibitors of thrombin-induced platelet aggregation (Table 5). It has been suggested that bothrojaracin and bothroalternin from *Bothrops jararaca* and *B. alternatus* venoms, respectively (75, 76) and triabin from *Triatoma pallidepennis* (77-79) bind to the exosite of thrombin preventing the interaction between thrombin and its receptors and hence platelet aggregation.

***Class V**: Other nonenzymatic inhibitors*. These inhibitors in this group inhibit platelet aggregation by currently unknown mechanism(s) (Table 6). Lebetins is a group of inhibitors isolated from *Vipera lebetina* snake venom (80). Lebetins 1 are shorter with 12 or 13 residues, whereas lebetins 2 have 37 or 38 residues. Lebetins 1 appear to be derived by proteolysis of their larger counterparts, lebetins 2. It would be interesting to determine whether lebetins, like disintegrins, are proteolytic products of a larger precursor protein. They inhibit platelet aggregation induced by thrombin, collagen and PAF-acether (80). They also inhibit fibrinogen-induced aggregation of chymotrypsin-treated platelets. Truncation of more than one residue at the amino terminal end results in the loss of activity (81). These short peptides strongly inhibit collagen-induced thrombocytopenia in rats (80, 81).

Inhibitors with enzymatic activity

Several proteins with enzymatic activity inhibit platelet aggregation. Some of these enzymes inhibit aggregation by indirect mechanisms either by the formation of a product, which inhibits platelet aggregation or by physical destruction of the agonist or its receptor. In these cases, the mechanisms appear to be simple and are directly dependent on the respective enzymatic activity. The study of such factors may not significantly contribute to our understanding of platelet aggregation.

Nucleotidases. ADP is a potent inducer of platelet aggregation that is secreted from the platelets as dense granules by various agonists. Logically, hydrolysis of ADP should lead to inhibition. Thus ADPases and 5'-nucleotidases inhibit platelet aggregation (Table 7). Such inhibitors have been isolated from *Trimeresurus gramineus* (82), *Agkistrodon acutus* (83) and *Vipera aspis* (84). It was concluded that the removal of ADP and possibly the generation of adenosine was responsible for the inhibitory effect (82).

Phospholipase A_2 enzymes. Some PLA_2 enzymes induce platelet aggregation, whereas others inhibit it. In addition, there are a few PLA_2 enzymes that both initiate as well as inhibit platelet aggregation, though under different conditions. However, not all PLA_2 enzymes affect platelet

aggregation. PLA_2 enzymes that affect platelet aggregation are classified into three major classes (85). Some of the inhibitors (Table 8) inhibit aggregation by mechanism(s) dependent on the enzymatic activity, whereas others appear to inhibit by mechanism(s) that are independent of their phospholipolytic activity. In the former case, the inhibitory effect may be due to the formation of lysophospholipids (86, 87). However, PLA_2 enzymes from *Naja nigricollis* (88), *Ophiophagus hannah* (89), *Pseudechis papuanus* (90), *Agkistrodon acutus* (91) and *A. halys* (92) seem to affect platelet aggregation inhibition by mechanisms independent of their enzymatic activity. The mechanism of antiplatelet effects of this group of PLA_2 enzymes is not yet understood.

Metalloproteinases. Some of the snake venom metalloproteinases inhibit platelet aggregation (Table 9). Most of these were first identified as fibrogenases, since they release peptides from the carboxy-terminal of fibrinogen. They are classified into α and β fibrinogenases based on their specificity for the Aα or Bβ chain of fibrinogen (93). α-Fibrinogenase inhibits platelet aggregation but not β-fibrinogenase. Since fibrinogen binding to its receptor GPIIb/IIIa complex is the essential final step in the platelet aggregation, it was initially thought that α-fibrinogenase inhibits platelet aggregation as a result of the depletion of fibrinogen or through the generation of plasma fibrinogen degradation products (94). However, direct testing of fibrinogen degradation products did not show significant inhibition (95). We showed that an α-fibrogenase from *Naja nigricollis* (96) inhibits platelet aggregation even in the absence of plasma fibrinogen. This was the first indication that fibrinogen may not be the primary target protein, which determines the antiplatelet effects, for these metalloproteinases. Several other studies also support this possibility. Kistomin, a novel α-fibrinogenase specifically cleaves GPIb resulting in the inhibition of platelet aggregation (97). Since metalloproteinases exist as large proteins, which are precursors of disintegrins and disintegrin-like polypeptides, these proteins could strongly inhibit platelet aggregation. Disintegrin/disintegrin-like domains in catrocollastatin, atrolysin A and atrolysin E inhibit platelet aggregation (60, 62, 98). Their precursors containing metalloproteinase domains also inhibit platelet aggregation. In catrocollastatin disintegrin-like domain determines its binding to collagen (62). Jararhagin, another metalloproteinase containing disintegrin-like domain, binds specifically to GPIa (64). Both these proteinases specifically inhibit collagen-induced platelet aggregation. Thus non-proteinase domains of metalloproteinases can significantly contribute to the recognition and binding to specific target proteins and hence their antiplatelet effects. The proteolytic activity of the metalloproteinase domain may also play an important role. For example, though jararhagin binds to GPIa, it cleaves the nearby GPIIa (64). The identification of new target proteins for the metalloproteinases and the structure-function relationships of these antiplatelet proteins could lead to development of novel antithrombotic agents.

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REFERENCES

1. Teng, CM, Huang, TF. Inventory of exogenous inhibitors of platelet aggregation. Thromb Haemost 1991; 65: 624-26.
2. Teng CM, Ma YH, Ouyang CH. Action mechanism of the platelet aggregation inducer and inhibitor from *Echis carinatus* snake venom. Biochim Biophys Acta 1985; 841: 8-14.
3. Ouyang CH, Ma YH, Jih HC, Teng CM. Characterization of the platelet aggregation inducer and inhibitor from *Echis carinatus* snake venom. Biochim Biophys Acta 1985, 841: 1-7.
4. Gan ZR, Gould RJ, Jacobs JW, Friedman PA, Polokoff MA. Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. J Biol Chem 1988; 263: 19827-32.
5. Huang TF, Holt JC, Kirby EP, Niewiarowski S. Trigramin: primary structure and its inhibition of von Willebrand factor binding to glycoprotein IIb/IIIa complex on human platelets. Biochemistry 1989; 28: 661-6.
6. Musial J, Niewiarowski S, Rucinski B, Stewart GJ, Cook JJ, Williams JA, Edmunds LH, Jr. Inhibition of platelet adhesion to surfaces of extracorporeal circuits by disintegrins: RGD-containing peptides from viper venoms. Circulation 1990; 82: 261-73.
7. Gould RJ, Polokoff MA, Friedman PA, Huang TF, Holt JC, Cook JJ, Niewiarowski S. Mini review. Disintegrins: a family of integrin inhibitory proteins from viper venoms. Proc Soc Exptl Biol Med 1990; 195: 168-71.
8. Kini RM, Evans HJ. Review article - Effects of snake venom proteins on blood platelets. Toxicon 1990; 28: 1387-422.
9. Kini RM, Evans HJ. Structural domains in venom proteins: evidence that metalloproteinases and nonenzymatic platelet aggregation inhibitors (disintegrins) from snake venoms are derived by proteolysis from a common precursor. Toxicon 1992; 30: 265-93.
10. Hite LA, Shannon JD, Bjarnason JB, Fox JW. Sequence of a cDNA clone encoding the zinc metalloproteinase hemorrhagic toxin ***e*** from *Crotalus atrox*: evidence for signal, zymogen, and disintegrin-like structures. Biochemistry 1992; 31: 6203-11.
11. Hite LA, Bjarnason JB, Fox JW. cDNA sequences for four snake venom metalloproteinases: structure, classification, and their relationship to mammalian reproductive proteins. Arch Biochem Biophys 1994; 308; 182-91.
12. Au LC, Chou JS, Chang KJ, Teh GW, Lin SB. Nucleotide sequence of a full-length cDNA encoding a common precursor of platelet aggregation inhibitor and hemorrhagic protein from *Calloselasma rhodostoma* venom. Biochim Biophys Acta 1993; 1173: 243-5.
13. Takeya H, Nishida S, Miyata T, Kawada SI, Saisaka Y, Morita T, Iwanaga S. Coagulation factor X activating enzyme from Russell's viper venom (RVV-X). A novel metalloproteinase with disintegrin (platelet aggregation inhibitor)-like and C-type lectin-like domains. J Biol Chem 1992; 267: 14109-17.
14. Takeya H, Nishida S, Nishino N, Makinose Y, Omori-Satoh T, Nikai T, Sugihara H, Iwanaga S. Primary structures of platelet aggregation inhibitors (disintegrins) autoproteolytically released from snake venom hemorrhagic metalloproteinases and new fluorogenic peptide substrates for these enzymes. J Biochem 1993; 113: 473-83.
15. Paine MJI, Desmond HP, Theakston RDG, Crampton JM. Purification, cloning, and molecular characterization of a high molecular weight hemorrhagic metalloproteinase, jararhagin, from *Bothrops jararaca* venom. Insights into the disintegrin gene family. J Biol Chem 1992; 267: 22869-76.

16. Paine MJI, Moura-de-Silva AM, Theakston RDG, Crampton JM. Cloning of metalloproteinase genes in the carpet viper (*Echis pyramidum leakeyi*). Further members of the metalloprotease/disintegrin gene family. Eur J Biochem 1994; 224: 483-8.
17. Nishida S, Fujita T, Kohno N, Atoda H, Morita T, Takeya H, Kido I, Paine MJI, Kawabata S, Iwanaga S. cDNA cloning and deduced amino acid sequence of prothrombin activator (ecarin) from Kenyan *Echis carinatus* venom. Biochemistry 1995; 34: 1771-8.
18. Yamada D, Shin Y, Morita T. Nucleotide sequence of a cDNA encoding a common precursor of disintegrin flavostatin and hemorrhagic factor HR2a from the venom of *Trimeresurus flavoviridis*. FEBS Lett 1999; 451: 299-302.
19. Selistre de Araujo HS, Ownby CL. Molecular cloning and sequence analysis of cDNAs for metalloproteinases from broad-banded copperhead *Agkistrodon contortrix laticinctus.* Arch Biochem Biophys 1995; 320: 141-8.
20. Scarborough RM, Rose JW, Hsu MA, Phillips DR, Fried VA, Campbell AM, Nannizzi L, Charo IF. Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of *Sistrurus m. barbouri*. J Biol Chem 1991; 266: 9359-62.
21. Oshikawa K, Terada S. Ussuristatin 2, a novel KGD-bearing disintegrin from *Agkistrodon ussuriensis* venom. J Biochem 1999; 125:31-5.
22. Scarborough RM, Naughton MA, Teng W, Rose JW, Phillips DR, Nannizzi L, Arfsten A, Campbell AM, Charo IF. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. J Biol Chem 1993; 268: 1066-73.
23. Adler M, Lazarus RA, Dennis MS, Wagner G. Solution structure of kistrin, a potent platelet aggregation inhibitor and GP IIb-IIIa antagonist. Science 1991; 253: 445-8.
24. Saudek V, Atkinson RA, Pelton JT. Three-dimensional structure of echistatin, the smallest active RGD protein. Biochemistry 1991; 30: 7369-72.
25. Cooke RM, Carter BG, Murray-Rust P, Hartshorn MJ, Herzyk P, Hubbard RE. The solution structure of echistatin: evidence for disulfide bond rearrangement in homologous snake toxins. Protein Engg 1992; 5: 473-7.
26. Senn H, Klaus W. The nuclear magnetic resonance solution structure of flavoridin, an antagonist of the platelet GP IIb-IIIa receptor. J Mol Biol 1993; 232: 907-25.
27. Smith KJ, Jaseja M, Lu X, Williams JA, Hyde EI, Trayer IP. Three-dimensional structure of the RGD-containing snake toxin albolabrin in solution, based on ^{1}H NMR spectroscopy and simulated annealing calculations. Int J Pept Protein Res 1996; 48: 220-8.
28. Lu X, Williams JA, Deadman JJ, Salmon GP, Kakkar VV, Wilkinson JM, Baruch D, Authi KS, Rahman S. Preferential antagonism of the interactions of the integrin $\alpha_{IIb}\beta_3$ with immobilized glycoprotein ligands by snake-venom RGD (Arg-Gly-Asp) proteins. Evidence supporting a functional role for the amino acid residues flanking the tripeptide RGD in determining the inhibitory properties of snake-venom RGD proteins. Biochem J 1994; 304: 929-36.
29. Rahman S, Lu X, Kakkar VV, Authi KS. The integrin $\alpha_{IIb}\beta_3$ contains distinct and interacting binding sites for snake-venom RGD (Arg-Gly-Asp) proteins. Evidence that the receptor-binding characteristics of snake-venom RGD proteins are related to the amino acid environment flanking the sequence RGD. Biochem J 1995; 312: 223-32.
30. Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. N Engl J Med 1995; 332:1553-9.

31. Lefkovits J, Topol EJ. Platelet glycoprotein IIb/IIIa receptor antagonists in coronary artery disease. Eur Heart J 1996; 17: 9-18.
32. Sheu JR, Lin CH, Huang TF. Triflavin, an antiplatelet peptide inhibits tumor cell-extracellular matrix adhesion via an RGD-dependent mechanism. J Lab Clin Med 1994; 123: 256-63.
33. Chiang HS, Yang RS, Huang TF. Arg-Gly-Asp containing peptide, rhodostomin, inhibits in vitro cell adhesion to extracellular matrices and platelet aggregation caused by Sao-2 human osteosarcoma cells. Br J Cancer 1995; 71: 265-70.
34. Sheu JR, Lin CH, Peng HC, Huang TF. Triflavin, an Arg-Gly-Asp containing peptide, inhibits the adhesion of tumor cells to matrix proteins via binding to multiple integrin receptors expressed on human hepatoma cells. Proc Soc Exp Biol Med 1996; 213: 71-9.
35. Sheu JR, Huang TF. Triflavin, an Arg-Gly-Asp containing peptide, inhibits B16F10 mouse melanoma cell adhesion to matrix proteins via direct binding to tumor cells. J Biomed Sci 1996; 3: 359-64.
36. Soszka T, Knudsen KA, Beviglia L, Rossi C, Poggi A. Niewiarowski S. Inhibition of murine melanoma cell-matrix adhesion and experimental metastasis by albolabrin, an RGD-containing peptide isolated from the venom of *Trimeresurus albolabris*. Exp Cell Res 1991; 196: 6-12.
37. Sheu JR, Lin CH, Chuang JL, Teng CM, Huang TF. Triflavin, an Arg-Gly-Asp containing peptide inhibits cell-substratum adhesion and melanoma cell-induced lung colonization. Jpn J Cancer Res 1992; 83: 885-93.
38. Morris VL, Schmidt EE, Koop S, MacDonald IC, Grattan Khokka R, McLane MA, Niewiarowski S, Chambers AF, Croom AC. Effects of disintegrin eristostatin on individual steps of hematogenous metastasis. Exp Cell Res 1995; 219: 571-8.
39. Trikha M, De Clerck YA, Markland FS. Contortrostatin, a snake venom disintegrin, inhibits α_1 integrin-mediated human metastatic melanoma cell adhesion and blocks experimental metastasis. Cancer Res 1994; 54: 4993-8.
40. Sheu JR, Lin CH, Chuang JL, Teng CM, Huang TF. Triflavin, an Arg-Gly-Asp containing peptide, inhibits aggregation of human platelets induced by human hepatoma cell line. Thrombos Res 1992; 66: 679-91.
41. Sheu JR, Yen MH, Kan YC, Hung WC, Chang PT, Luk HN. Inhibition of angiogenesis in vitro and in vivo: comparison of the relative activities of triflavin, an Arg-Gly-Asp-containing peptide and anti-$\alpha_v\beta_3$ integrin monoclonal antibody. Biochim Biophys Acta 1997; 1336: 445-54.
42. Yeh CH, Peng HC, Huang TF. Accutin, a new disintegrin, inhibits angiogenesis in vitro and in vivo by acting as integrin $\alpha_v\beta_3$ antagonist and inducing apoptosis. Blood 1998; 92: 3268-76.
43. Kumar CC, Nie H, Rogers CP, Malkowski M, Maxwell E, Catino JJ, Armstrong L. Biochemical characterization of the binding of echistatin to integrin $\alpha_v\beta_3$ receptor. J Pharmacol Exp Ther 1997; 283: 843-53.
44. Dresner Pollak R, Rosenblatt M. Blockade of osteoclast-mediated bone resorption through occupancy of the integrin receptor: a potential approach to the therapy of osteoporosis. J Cell Biochem 1994; 56: 323-30.
45. Yamamoto M, Fisher JE, Gentile M, Seedor JG. Leu CT, Rodan SB, Rodan GA. The integrin ligand echistatin prevents bone loss in ovariectomized mice and rats. Endocrinology 1998; 139: 1411-9.

46. Masarachia P, Yamamoto M, Leu CT, Rodan G, Duong L. Histomorphometric evidence for echistatin inhibition of bone resorption in mice with secondary hyperparathyroidism. Endocrinology 1998; 139: 1401-10.
47. McDowell RS, Dennis MS, Louie A, Shuster M, Mulkerrin MG, Lazarus RA. Mambin, a potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. Biochemistry 1992; 31: 4766-72.
48. Jaseja M, Lu X, Williams JA, Sutcliffe MJ, Kakkar VV, Parslow RA, Hyde EI. 1H-NMR assignments and secondary structure of dendroaspin, an RGD-containing glycoprotein IIb-IIIa ($\alpha_{IIb}\beta_3$) antagonist with a neurotoxin fold. Eur J Biochem 1994; 226: 861-8.
49. Seymour JL, Henzel WJ, Nevins B, Stults JT, Lazarus RA. Decorsin. A potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor from the leech *Macrobdella decora.* J Biol Chem 1990; 265: 10143-7.
50. Mazur P, Henzel WJ, Seymour JL, Lazarus RA. Ornatins: potent glycoprotein IIb-IIIa antagonists and platelet aggregation inhibitors from the leech *Placobdella ornata*. Eur J Biochem 1991; 202: 1073-82.
51. Wang X, Coons LB, Taylor DB, Stevens SE Jr, Gartner TK. Variabilin, a novel RGD-containing antagonist of glycoprotein IIb-IIIa and platelet aggregation inhibitor from the hard tick *Dermacentor variabilis*. J Biol Chem 1996; 271: 17785-90.
52. Karczewski J, Endris R, Connolly TM. Disagregin is a fibrinogen receptor antagonist lacking the Arg-Gly-Asp sequence from the tick, *Ornithodoros moubata.* J Biol Chem 1994; 269: 6702-8.
53. Girma J-P, Meyer D, Verweij CL, Pannekoek H, Sixma JJ. Structure-function relationship of human von Willebrand factor. Blood 1987; 70: 605-11.
54. Sadler JE. von Willebrand factor. J Biol Chem 1991; 266: 18172-8.
55. Taniuchi Y, Kawasaki T, Fujimura Y, Suzuki M, Titani K, Sakai Y, Kaku S, Hisamichi N, Satoh N, Takenaka T, et al Flavocetin-A and -B, two high molecular mass glycoprotein Ib binding proteins with high affinity purified from *Trimeresurus flavoviridis* venom, inhibit platelet aggregation at high shear stress. Biochim Biophys Acta1995; 1244: 331-8.
56. Peng M, Holt JC, Niewiarowski S. Isolation, characterization and amino acid sequence of echicetin α subunit, a specific inhibitor of von Willebrand factor and thrombin interaction with glycoprotein Ib. Biochem Biophys Res Commun1994; 205: 68-72.
57. Peng M, Lu W, Beviglia L, Niewiarowski S, Kirby EP. Echicetin: a snake venom protein that inhibits binding of von Willebrand factor and alboaggregins to platelet glycoprotein Ib. Blood. 1993; 81: 2321-8.
58. Sixma JJ, van Zanten GH, Saelman EU, Verkleij M, Lankhof H, Nieuwenhuis HK, de Groot PG. Platelet adhesion to collagen. Thromb Haemost 1995; 74: 454-9.
59. Shimokawa K, Shannon JD, Jia LG, Fox JW. Sequence and biological activity of catrocollastatin-C: a disintegrin-like/cysteine-rich two-domain protein from *Crotalus atrox* venom. Arch Biochem Biophys 1997; 343: 35-43.
60. Jia LG, Wang XM, Shannon JD, Bjarnason JB, Fox JW. Function of disintegrin-like/cysteine-rich domains of atrolysin A. Inhibition of platelet aggregation by recombinant protein and peptide antagonists. J. Biol Chem 1997; 272: 13094-102.
61. Usami Y, Fujimura Y, Miura S, Shima H, Yoshida E, Yoshioka A, Hirano K, Suzuki M, Titani K. A 28 kDa-protein with disintegrin-like structure (jararhagin-C) purified from *Bothrops jararaca* venom inhibits collagen- and ADP-induced platelet aggregation. Biochem Biophys Res Commun 1994; 201: 331-9.

62. Zhou Q, Dangelmaier C, Smith JB. The hemorrhagin catrocollastatin inhibits collagen-induced platelet aggregation by binding to collagen via its disintegrin-like domain. Biochem Biophys Res Commun 1996; 19: 720-6.
63. Kamiguti AS, Moura da Silva AM, Laing GD, Knapp T, Zuzel M, Crampton JM, Theakston RD. Collagen-induced secretion-dependent phase of platelet aggregation is inhibited by the snake venom metalloproteinase jararhagin. Biochim Biophys Acta 1997; 1335: 209-17.
64. Kamiguti AS, Hay CR, Zuzel M. Inhibition of collagen-induced platelet aggregation as the result of cleavage of $\alpha_2\beta_1$-integrin by the snake venom metalloproteinase jararhagin. Biochem J 1996; 320: 635-41.
65. Liu CZ, Huang TF. Crovidisin, a collagen-binding protein isolated from snake venom of *Crotalus viridis*, prevents platelet-collagen interaction. Arch Biochem Biophys 1997; 337: 291-9.
66. Munro R, Jones CP, Sawyer RT. Calin - a platelet adhesion inhibitor from the saliva of the medicinal leech. Blood Coagul Fibrinolysis 1991; 2: 179-84.
67. Connolly TM, Jacobs JW, Condra C. An inhibitor of collagen-stimulated platelet activation from the salivary glands of the *Haementeria officinalis* leech. I. Identification, isolation, and characterization. J Biol Chem 1992; 267: 6893-8.
68. Keller PM, Schultz LD, Condra C, Karczewski J, Connolly TM. An inhibitor of collagen-stimulated platelet activation from the salivary glands of the *Haementeria officinalis* leech. II. Cloning of the cDNA and expression. J Biol Chem 1992; 267: 6899-904.
69. Waxman L, Connolly TM. Isolation of an inhibitor selective for collagen-stimulated platelet aggregation from the soft tick *Ornithodoros moubata.* J Biol Chem 1993; 268: 5445-9.
70. Keller PM, Waxman L, Arnold BA, Schultz LD, Condra C, Connolly TM. Cloning of the cDNA and expression of moubatin, an inhibitor of platelet aggregation. J Biol Chem 1993; 268: 5450-6.
71. Karczewski J, Waxman L; Endris RG, Connolly TM. An inhibitor from the argasid tick *Ornithodoros moubata* of cell adhesion to collagen. Biochem Biophys Res Commun 1995; 208: 532-41.
72. Noeske-Jungblut C, Krätzschmar J, Haendler B, Alagon A, Possani L, Verhallen P, Donner P, Schleuning WD. An inhibitor of collagen-induced platelet aggregation from the saliva of *Triatoma pallidipennis.* J Biol Chem 1994; 269: 5050-3.
73. Haendler B, Becker A, Noeske-Jungblut C, Krätzschmar J, Donner P, Schleuning WD. Expression of active recombinant pallidin, a novel platelet aggregation inhibitor, in the periplasm of *Escherichia coli*. Biochem J 1995; 307: 465-70.
74. Fenton JW, Ofosu FA, Moon DG Maraganore JM. Thrombin structure and function: why thrombin is the primary target for antithrombotics. Blood Coagul Fibrinolysis 1991; 2: 69-75.
75. Zingali RB, Jandrot Perrus M, Guillin MC, Bon C. Bothrojaracin, a new thrombin inhibitor isolated from *Bothrops jararaca* venom: characterization and mechanism of thrombin inhibition. Biochemistry 1993; 32: 10794-802.
76. Castro HC, Dutra DL, Oliveira Carvalho AL, Zingali RB. Bothroalternin, a thrombin inhibitor from the venom of *Bothrops alternatus.* Toxicon 1998; 36: 1903-12.
77. Noeske Jungblut C, Haendler B, Donner P, Alagon A, Possani L, Schleuning WD. Triabin, a highly potent exosite inhibitor of thrombin. J Biol Chem 1995; 270: 28629-34.

78. Fuentes Prior P Noeske Jungblut C Donner P Schleuning WD Huber R, Bode W. Structure of the thrombin complex with triabin, a lipocalin-like exosite-binding inhibitor derived from a triatomine bug. Proc Natl Acad Sci U S A 1997; 94: 11845-50.
79. Glusa E, Bretschneider E, Daum J, Noeske Jungblut C. Inhibition of thrombin-mediated cellular effects by triabin, a highly potent anion-binding exosite thrombin inhibitor. Thromb Haemost 1997; 77: 1196-200.
80. Barbouche R, Marrakchi N, Mansuelle P, Krifi M, Fenouillet E, Rochat H, El Ayeb M. Novel anti-platelet aggregation polypeptides from *Vipera lebetina* venom: isolation and characterization. FEBS Lett 1996; 392: 6-10.
81. Barbouche R, Marrakchi N, Mabrouk K, Krifi MN, Van Rietschoten J, Fenouillet E, El Ayeb M, Rochat H. Anti-platelet activity of the peptides composing the lebetin 1 family, a new class of inhibitors of platelet aggregation. Toxicon 1998; 36: 1939-47.
82. Ouyang C, Huang TF. Inhibition of platelet aggregation by 5'-nucleotidase purified from *Trimeresurus gramineus* snake venom. Toxicon 1983; 21: 491-501.
83. Ouyang C, Huang TF. Platelet aggregation inhibitors from *Agkistrodon acutus* snake venom. Toxicon 1986; 24: 1099-106.
84. Boffa MC, Boffa GA. Correlations between the enzymatic and factors active on blood coagulation and platelet aggregation from the venom of *Vipera aspis.* Biochim Biophys Acta 1974; 354: 275-90.
85. Kini RM, Evans HJ. Effects of phospholipase A_2 enzymes on platelet aggregation. In: Venom Phospholipase A_2 Enzymes: Structure, Function and Mechanism. Kini RM, ed. Chichester: John Wiley 1997; 369-387.
86. Teng CM, Kuo YP, Lee LG, Ouyang C. Effect of cobra venom phospholipase A_2 on platelet aggregation in comparison with those produced by arachidonic acid and lysophosphatidylcholine. Thromb Res 1986; 44: 875-86.
87. Yuan Y, Jackson SP, Newnham HH, Mitchell CA, Salem HH. An essential role for lysophosphatidylcholine in the inhibition of platelet aggregation by secretory phospholipase A_2. Blood 1995; 86: 4166-74.
88. Kini RM, Evans HJ. Correlation between the enzymatic activity, anticoagulant and antiplatelet effects of phospholipase A_2 isoenzymes from *Naja nigricollis* venom. Thromb Haemost1988; 60: 170-3.
89. Huang MZ, Gopalakrishnakone P, Kini RM. Role of enzymatic activity in the antiplatelet effects of a phospholipase A_2 from *Ophiophagus hannah* snake venom. Life Sci 1997; 61: 2211-7.
90. Laing GD, Kamiguti AS, Wilkinson MC, Lowe GM, Theakston RD. Characterisation of a purified phospholipase A_2 from the venom of the Papuan black snake (*Pseudechis papuanus*). Biochim Biophys Acta 1995; 1250: 137-43.
91. Chen RH, Chen YC. Isolation of an acidic phospholipase A_2 from the venom of *Agkistrodon acutus* (five pace snake) and its effect on platelet aggregation. Toxicon 1989; 27: 675-82.
92. Ouyang C, Yeh HI, Huang TF. A potent platelet aggregation inhibitor purified from *Agkistrodon halys* (mamushi) snake venom. Toxicon 1983; 21: 797-804.
93. Ouyang C, Teng CM. Fibrinogenolytic enzymes of *Trimeresurus* venom. Biochim Biophys Acta 1976; 420: 298-308.

94. Boffa MC, Boffa GA. Correlation between the enzymatic activities and the factors active on blood coagulation and platelet aggregation from the venom of *Vipera aspis*. Biochim Biophys Acta 1974; 354: 275-90.
95. Ouyang C, Teng CM, Chen YC. Properties of fibrinogen degradation products produced by α and β fibrinogenases of *Trimeresurus mucrosquamatus* snake venom. Toxicon 1979; 17: 121-6.
96. Kini RM, Evans HJ. Inhibition of platelet aggregation by a fibrinogenase from *Naja nigricollis* venom is independent of fibrinogen degradation. Biochim Biophys Acta 1991; 1095: 117-21.
97. Huang TF, Chang MC, Teng CM. Antiplatelet protease, kistomin, selectively cleaves human platelet glycoprotein Ib. Biochim Biophys Acta1993; 1158: 293-9.
98. Shimokawa K, Jia LG, Shannon JD, Fox JW. Isolation, sequence analysis, and biological activity of atrolysin E/D, the non-RGD disintegrin domain from *Crotalus atrox* venom. Arch Biochem Biophys 1998; 354: 239-46.
99. Yeh CH, Peng HC, Yih JB, Huang TF. A new short chain RGD-containing disintegrin, accutin, inhibits the common pathway of human platelet aggregation. Biochim Biophys Acta 1998; 1425: 493-504.
100. Williams J, Rucinski B, Holt J, Niewiarowski S. Elegantin and albolabrin purified peptides from viper venoms: homologies with the RGDS domain of fibrinogen and von Willebrand factor. Biochim Biophys Acta 1990; 1039:81-89.
101. Chao BH; Jakubowski JA; Savage B; Chow EP; Marzec UM; Harker *Agkistrodon piscivorus piscivorus* platelet aggregation inhibitor: a potent inhibitor of platelet activation. Proc Natl Acad Sci U S A 1989; 86: 8050-4.
102. Savage B, Marzec UM, Chao BH, Harker LA, Maraganore JM, Ruggeri ZM. Binding of the snake venom-derived proteins applaggin and echistatin to the arginine-glycine-aspartic acid recognition site(s) on platelet glycoprotein IIb-IIIa complex inhibits receptor function. J Biol Chem 1990; 265: 11766-72.
103. Huang TF, Wang WJ, Teng CM, Liu CS, Ouyang C. Purification and characterization of an antiplatelet peptide, arietin, from *Bitis arietans* venom. Biochim Biophys Acta 1991; 1074: 136-43.
104. Huang TF, Wang WJ, Teng CM, Ouyang C. Mechanism of action of the antiplatelet peptide, arietin, from *Bitis arietans* venom. Biochim Biophys Acta 1991: 1074: 144-50.
105. Rucinski B, Niewiarowski S, Holt JC, Soszka T, Knudsen KA. Batroxostatin, an Arg-Gly-Asp-containing peptide from *Bothrops atrox,* is a potent inhibitor of platelet aggregation and cell interaction with fibronectin. Biochim Biophys Acta 1990; 1054: 257-62.
106. Dennis MS, Henzel WJ, Pitti RM, Lipari MT, Napier MA, Deisher, TA, Bunting S, Lazarus, RA. Platelet glycoprotein IIb-IIIa protein antagonists from snake venoms: Evidence for a family of platelet-aggregating inhibitors. Proc Natl Acad Sci USA 1990, 87:2471-5.
107. Shebuski RJ, Ramjit DR, Bencen GH, Polokoff MA. Characterization and platelet inhibitory activity of bitistatin, a potent arginine-glycine-aspartic acid-containing peptide from the venom of the viper *Bitis arietans*. J Biol Chem 1989; 264: 21550-6.
108. Marrakchi N, Barbouche R, Bon C, El Ayeb M. Cerastatin, a new potent inhibitor of platelet aggregation from the venom of the Tunisian viper, *Cerastes cerastes*. Toxicon 1997; 35: 125-35.
109. Liu CZ, Peng HC, Huang TF. Crotavirin, a potent platelet aggregation inhibitor purified from the venom of the snake *Crotalus viridis.* Toxicon 1995, 33: 1289-98.

110. McLane MA, Kowalska MA, Silver L, Shattil SJ, Niewiarowski S. Interaction of disintegrins with the $\alpha_{IIb}\beta_3$ receptor on resting and activated human platelets. Biochem J 1994; 301: 429-36.
111. McLane MA, Gabbeta J, Rao AK, Beviglia L, Lazarus RA, Niewiarowski S. A comparison of the effect of decorsin and two disintegrins, albolabrin and eristostatin, on platelet function. Thromb Haemost 1995; 74: 1316-22.
112. Kawasaki T, Sakai Y, Taniuchi Y, Sato K, Maruyama K, Shimizu M, Kaku S, Yano S, Inagaki O, Tomioka K, Yanagisawa I, Takenaka T. Biochemical characterization of a new disintegrin, flavostatin, isolated from *Trimeresurus flavoviridis* venom. Biochimie 1996; 78: 245-52.
113. Maruyama K, Kawasaki T, Sakai Y, Taniuchi Y, Shimizu M, Kawashima H, Takenaka T. Isolation and amino acid sequence of flavostatin, a novel disintegrin from the venom of *Trimeresurus flavoviridis.* Peptides1997; 18: 73-8.
114. Huang TF, Peng HC, Peng IS, Teng CM, Ouyang C. An antiplatelet peptide, gabonin, from *Bitis gabonica* snake venom. Arch Biochem Biophys 1992; 298: 13-20.
115. Huang TF, Liu CZ, Ouyang CH, Teng CM. Halysin, an antiplatelet Arg-Gly-Asp-containing snake venom peptide, as fibrinogen receptor antagonist. Biochem Pharmacol 1991; 42: 1209-19.
116. Trikha M, Rote WE, Manley PJ, Lucchesi BR, Markland FS. Purification and characterization of platelet aggregation inhibitors from snake venoms. Thromb Res 1994, 73: 39-52.
117. Kang IC, Chung KH, Lee SJ, Yun Y, Moon HM, Kim DS. Purification and molecular cloning of a platelet aggregation inhibitor from the snake (*Agkistrodon halys brevicaudus*) venom. Thromb Res 1998; 91: 65-73.
118. Park D, Kang I, Kim H, Chung K, Kim DS, Yun Y. Cloning and characterization of novel disintegrins from *Agkistrodon halys* venom. Mol Cells 1998; 8: 578-84.
119. Huang TF, Sheu JR, Teng CM. A potent antiplatelet peptide, triflavin, from *Trimeresurus flavoviridis* snake venom. Biochem J 1991; 277: 351-7.
120. Huang TF, Sheu JR, Teng CM, Chen SW; Liu CS. Triflavin, an antiplatelet Arg-Gly-Asp-containing peptide, is a specific antagonist of platelet membrane glycoprotein IIb-IIIa complex. J Biochem 1991; 109: 328-34.
121. Huang TF, Holt JC, Lukasiewicz H, Niewiarowski S. Trigramin. A low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. J Biol Chem 1987; 262: 16157-63.
122. Tsai IH, Wang YM, Lee YH. Characterization of a cDNA encoding the precursor of platelet aggregation inhibition and metalloproteinase from *Trimeresurus mucrosquamatus* venom. Biochim Biophys Acta 1994; 1200: 337-40.
123. Huang TF, Wu YJ, Ouyang C. Characterization of a potent platelet aggregation inhibitor from *Agkistrodon rhodostoma* snake venom. Biochim Biophys Acta 1987; 925: 248-57.
124. Grevelink SA, Youssef DE, Loscalzo J, Lerner EA. Salivary gland extracts from the deerfly contain a potent inhibitor of platelet aggregation. Proc Natl Acad Sci U S A1993; 90: 9155-8.
125. Chen YL, Tsai IH. Functional and sequence characterization of agkicetin, a new glycoprotein Ib antagonist isolated from *Agkistrodon acutus* venom. Biochem Biophys Res Commun 1995; 210: 472-7.

126. Scarborough RM, Hsu MA, Teng W, Nannizzi L, Rose JW. Purification and characterization of potent inhibitors of von Willebrand factor binding to glycoprotein (GP)1b from the venom of timber rattlesnake, *Crotalus h. horridus.* Blood 1991; 78:1568 (abstract).
127. Fujimura Y, Ikeda Y, Miura S, Yoshida E, Shima H, Nishida S, Suzuki M, Titani K, Taniuchi Y, Kawasaki T. Isolation and characterization of jararaca GPIb-BP, a snake venom antagonist specific to platelet glycoprotein Ib. Thromb Haemost 1995; 74: 743-50.
128. Kawasaki T, Fujimura Y, Usami Y, Suzuki M, Miura S, Sakurai Y, Makita K, Taniuchi Y, Hirano K, Titani K. Complete amino acid sequence and identification of the platelet glycoprotein Ib-binding site of jararaca GPIb-BP, a snake venom protein isolated from *Bothrops jararaca.* J Biol Chem 1996; 271: 10635-9.
129. Kawasaki T, Taniuchi Y, Hisamichi N, Fujimura Y, Suzuki M, Titani K, Sakai Y, Kaku S, Satoh N, Takenaka T, et al Tokaracetin, a new platelet antagonist that binds to platelet glycoprotein Ib and inhibits von Willebrand factor-dependent shear-induced platelet aggregation. Biochem J 1995; 308: 947-53.
130. Chow G, Subburaju S, Kini RM. Purification, characterization, and amino acid sequence determination of acanthins, potent inhibitors of platelet aggregation from *Acanthophis antarcticus* (common death adder) venom. Arch Biochem Biophys 1998; 354: 232-8.
131. Sim KL. Purification and preliminary characterisation of praelongin phospholipases, antiplatelet agents from the snake venom of *Acanthophis praelongus*. Biochim Biophys Acta 1998; 1379: 198-206.
132. Huang TF, Yeh HI, Ouyang C. Mechanism of action of the platelet aggregation inhibitor purified from *Agkistrodon halys* (mamushi) snake venom. Toxicon 1984; 22: 243-51.
133. Chen YC, Maraganore JM, Reardon I, Heinrikson RL. Characterization of the structure and function of three phospholipases A_2 from the venom of *Agkistrodon halys pallas*. Toxicon. 1987; 25: 401-9.
134. Yuan Y, Jackson SP, Mitchell CA, Salem HH. Purification and characterisation of a snake venom phospholipase A_2: a potent inhibitor of platelet aggregation. Thromb Res 1993; 70: 471-81.
135. Subburaju S, Kini RM. Isolation and purification of superbins I and II from *Austrelaps superbus* (copperhead) snake venom and their anticoagulant and antiplatelet effects. Toxicon 1997; 35: 1239-50.
136. Kemparaju K, Krishnakanth TP, Gowda TV. Purification and characterization of a platelet aggregation inhibitor acidic phospholipase A_2 from Indian saw-scaled viper (*Echis carinatus*) venom. Toxicon 1999; 37: 1659-71.
137. Fuly AL, Machado OL, Alves EW, Carlini CR. Mechanism of inhibitory action on platelet activation of a phospholipase A_2 isolated from *Lachesis muta* (Bushmaster) snake venom. Thromb Haemost 1997; 78: 1372-80.
138. Fuly AL, Machado OL, Alves EW, Carlini CR. Mechanism of inhibitory action on platelet activation of a phospholipase A_2 isolated from *Lachesis muta* (Bushmaster) snake venom. Thromb Haemost 1997; 78: 1372-80.
139. Ouyang C, Huang TF. Potent platelet aggregation inhibitor from *Trimeresurus gramineus* snake venom. Biochim Biophys Acta 1983; 757: 332-41.
140. Huang TF, Ouyang C. Action mechanism of the potent platelet aggregation inhibitor from *Trimeresurus gramineus* snake venom. Thromb Res 1984; 33: 125-38.

141. Prasad BN, Kemparaju K, Bhatt KG, Gowda TV. A platelet aggregation inhibitor phospholipase A_2 from Russell's viper (*Vipera russelli*) venom: isolation and characterization. Toxicon 1996; 34: 1173-85.
142. Teng CM, Chen YH, Ouyang C. Biphasic effect on platelet aggregation by phospholipase A purified from *Vipera russelli* snake venom. Biochim Biophys Acta 1984; 772: 393-402.
143. Li YS, Liu KF, Wang QC, Ran YL, Tu GC. A platelet function inhibitor purified from *Vipera russelli siamensis* (Smith) snake venom. Toxicon 1985; 23: 895-903.
144. Li YS, Liu KF, Wang QC. Mechanism of action of the platelet function inhibitor from *Vipera russelli siamensis* snake venom. Toxicon 1986; 24: 875-83.
145. Ouyang C, Huang TF. Effect of the purified phospholipases A_2 from snake and bee venoms on rabbit platelet function. Toxicon 1984; 22: 705-18.
146. Huang TF, Chiang HS. Effect on human platelet aggregation of phospholipase A_2 purified from *Heloderma horridum* (beaded lizard) venom. Biochim Biophys Acta 1994; 1211: 61-8.
147. Ouyang C, Hwang LH, Huang TF. α-Fibrogenase from *Agkistrodon rhodostoma* (Malayan pit viper) snake venom. Toxicon 1983; 21: 25-33.
148. Ouyang C, Hwang LJ, Huang TF. Inhibition of rabbit platelet aggregation by a-fibrogenase purified from *Calloselasma rhodostoma* (Malayan pit viper) venom. J Formosan Med Assoc 1985; 84: 1197-206.
149. Ouyang C, Teng CM, Chen YC. Physicochemical properties of α and β fibrinogenases of *Trimeresurus mucrosquamatus* venom. Biochim Biophys Acta 1977; 481: 622-30.
150. Teng CM, Ouyang C, Lin SC. Species difference in the fibrinogenolytic effects of α and β fibrinogenases from *Trimeresurus mucrosquamatus* snake venom. Toxicon 1985; 23: 777-82.
151. Gasmi A, Karoui M, Benlasfar Z, Karoui H, el Ayeb M, Dellagi K. Purification and characterization of a fibrinogenase from *Vipera lebetina* (desert adder) venom. Toxicon 1991; 29: 827-36.
152. Gasmi A, guermazi S, Chabchoub A, Makni K, Karoui H, Elayeb M, Dellagi K. Effect of a fibrinogenase from *Vipera lebetina* venom on human blood coagulation factors and platelets. Toxicon 1993; 31: 520.
153. Kamiguti AS, Slupsky JR, Zuzel M, Hay CR. Properties of fibrinogen cleaved by Jararhagin, a metalloproteinase from the venom of *Bothrops jararaca.* Thromb Haemost 1994; 72: 244-9.
154. Huang TF, Chang MC, Peng HC, Teng CM. A novel alpha-type fibrinogenase from *Agkistrodon rhodostoma* snake venom. Biochim Biophys Acta 1992; 1160: 262-8.
155. Fujimura S, Rikimara T, Baba S, Hori J, Hao XQ, Terada S, Kimoto E. Purification and characterization of a non-hemorrhagic metalloproteinase from *Agkistrodon halys brevicaudus* venom. Biochim Biophys Acta 1995; 1243: 94-100.
156. Evans HJ. Purification and properties of a fibrinogenase from the venom of *Naja nigricollis.* Biochim Biophys Acta 1984; 802: 49-54.
157. Zhou Q, Smith JB, Grossman MH. Molecular cloning and expression of catrocollastatin, a snake-venom protein from *Crotalus atrox* (western diamondback rattlesnake) which inhibits platelet adhesion to collagen. Biochem J 1995; 307: 411-7.

158. Kini RM, Caldwell RA, Wu QY, Baumgarten CM, Feher JJ, Evans HJ. Flanking proline residues identify the L-type Ca^{2+} channel binding site of calciseptine and FS2. Biochemistry 1998; 37: 9058-63.

Source	Protein	Chain	1	45
Bitis arietans	Bitistatin	Long	SPPVCGNEIL EQGEDCDCGS PANCQDQCCN AATCKLTPGS QCNHG	
Bitis arietans	Bitan α	Long	SPPVCGNKIL EQGEDCDCGS PANCQDRCCN AATCKLTPGS QCNYG	
Trimeresurus gramineus	Trigramin	Medium	EAGEDCDCGS PAN PCCD AATCKLIPGA QCGEG	
Agkistrodon rhodostoma	Kistrin	Medium	GKECDCSS PEN PCCD AATCKLRPGA QCGEG	
Echis carinatus	Echistatin	Short	<ECESG	
Eristocophis macmahoni	Eristocophin	Short	<ERQEE PCATG	

Source	Protein	Chain	46 *** 80
Bitis arietans	Bitistatin	Long	ECCDQ CKFKKARTVC RIARGDWNDD YCTGKSSDCP WNH
Bitis arietans	Bitan α	Long	ECCDQ CRFKKAGTVC RIARGDWNDD YCTGKSSDCP WNH
Trimeresurus gramineus	Trigramin	Medium	LCCDQ CSFIEEGTVC RIARGDDLDD YCNGRSAGCP RNPFH
Agkistrodon rhodostoma	Kistrin	Medium	LCCEQ CKFSRAGKIC RIPRGDMPDD RCTGQSADCP RYH
Echis carinatus	Echistatin	Short	PCCRN CKFLKEGTIC KRARGDDMDD YCNGKTCDCP RNPHKGP
Eristocophis macmahoni	Eristocophin	Short	PCCRR CKFKRAGKVC RVARGDWNDD YCTGKSCDCP RNPWNG

Figure 1. Amino acid sequences of disintegrins. Only a few selected sequences are shown. The amino acid sequences are taken from the following references: Bitistatin (107), bitan α (106), trigramin (5), kistrin (106), echistatin (4) and eristocophin (20). The one-letter notation of amino acids is used. Blank spaces indicate deletions. <E represents 5-pyrrolidone-2-carboxylic acid (pyroglutamyl residue). The RGD sequence in the binding site of disintegrins is shown by asterisk.

Species	Protein	Biological Activity	1 25
D. j. kaimosae	Mambin[a]	Aggregation Inhibitor	RICYNHLGTK PPTTETCQED SCYKN
D. p. polylepis	Toxin α	Postsynaptic Neurotoxin	RICYNHQSTT RATTKSCEEN SCYKK
D. j. kaimosae	V^{I}_{n} 1	Postsynaptic Neurotoxin	RICYNHQSTT PATTKSCGEN SCYKK
D. p. polylepis	Calciseptine	Ca^{2+} Channel Blocker	RICYIHKASL PRATKTCVEN TCYKM
D. p. polylepis	FS2	Ca^{2+} Channel Blocker	RICYSHKASL PRATKTCVEN TCYKM
D. angusticeps	Toxin C	Anti-acetylcholinesterase	TICYSHTTTS RAILKDCGEN SCYRK
D. p. polylepis	Fasciculin	Anti-acetylcholinesterase	TMCYSHTTTS RAILTNCGEN SCYRK

Species	Protein	26 62
D. j. kaimosae	Mambin[a]	IWTFD N IIRRGCG CFTPRGDMPG PYCCES DKC NL
D. p. polylepis	Toxin α	YWRDH RGTIIERGCG CPKVKPGVGI H CCQS DKC NY
D. j. kaimosae	V^{I}_{n} 1	TWSDH RGTIIERGCG CPKVKQGIHL H CCQS DKC NY
D. p. polylepis	Calciseptine	FIRTQ REYISERGCG CPTAMWPYQT E CCKG DRC NK
D. p. polylepis	FS2	FIRTH REYISERGCG CPTAMWPYQT E CCKG DRC NK
D. angusticeps	Toxin C	SRRHP PKMVLGRGCG CPPGDDYLEV K CCTSPDKC NY
D. p. polylepis	Fasciculin	SRRHP PKMVLGRGCG CPPGDDYLEV K CCTSPDKC NY

Figure 2. Structural comparison of mambin with related toxins from mamba venoms. The original references for each sequence are given in Kini et al. (158). * Mambin and dendroaspin have identical amino acid sequences. The one-letter notation of amino acids is used. Blank spaces indicate deletions.

Protein	Species	
(a) Platelet aggregation inhibitors from leeches		
Decorsin	*Macrobdella decora*	APRLPQCQG DDQEKCLCNK DECPPGQCRF PRGDADPYCE
Ornatin B	*Placobdella ornata*	IYVRPTNDEL NYCGDFRELG QPDKKCRCDG KPCTVGRCKF ARGDNDDKCI SA
Ornatin C	*Placobdella ornata*	IYVRPTKDEL LYCGEFRELG QPDKKCRCDG KPCTVGRCKF ARGDADDKCT SA
Ornatin E	*Placobdella ornata*	IYVRPTKDEL LYCGEFRELG QPDKKCRCDG KPCTVGRCNF ARGDNDDKCI
(b) Platelet aggregation inhibitors from ticks		
Variabilin	*Dermacentor variabilis*	NTFSDENPGF PCDCTSADAK RACGIQCACW PRGDTPGGGR RIIDGQQ
Disagregin	*Ornithodoros moubata*	SDDKCQGRPM YGCREDDDSV FGWTYDSNHG QCWKGSYCKH RRQPSNYFAS QQECRNTCGA

Figure 3. Amino acid sequences of fibrinogen receptor antagonists isolated from leeches and ticks. The amino acid sequences are taken from the following references: Decorsin (49), ornatins (50), variabilin (51) and disagregin (52). The one-letter notation of amino acids is used.

Table 1. Class I. Disintegrins and related antagonists of fibrinogen receptor glycoprotein IIb/IIIa complex (GPIIb/IIIa)

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Accutin	*Agkistrodon acutus*	5241 Da, 47aa	66-267 nM	99
Agkistrostatin	*Agkistrodon piscivorus*			7
Albolabrin	*Trimeresurus albolabris*	7574 Da, 73 aa, single chain, pI 4.27	220 nM	100
Applaggin	*Agkistrodon piscivorus piscivorus*	17 700 Da, 71 aa, a disulfide linked homodimer	12-128 nM	101, 102
Arietin /Bitistatin	*Bitis arietans*	8500 Da	130-270 nM	103, 104
Barbourin	*Sistrurus m. barbouri*	73 aa	309 nM	20
Batroxostatin	*Bothrops atrox*	71 aa	133 nM	105
Bitan _	*Bitis arietans*	8987 Da, 83aa	108 nM	106
Bitistatin	*Bitis arietans*	9022 Da, 83aa, single chain	237 nM	107
Carinatin	*Echis carinatus*	6 800 Da, acidic glycoprotein, 22.1 % neutral sugars, pI 4.8	1.47 nM	2, 3
Cerastatin	*Cerastes cerastes*	32 000 Da, pI 6.2, glycoprotein, oligometric structure	2.3 nM	108
Contortrostatin	*Agkistrodon contortrix contortrix*	15 000 Da, homodimer, 13 500 Da	49 – 1 150 nM for platelet of different species	14
Crotavirin	*Crotalus viridis*	9200 Da, single chain	110 nM	109
Echistatin α1	*Echis carinatus*	5400 Da, 49aa, pI 8.3	30 nM	106
Echistatin α2	*Echis carinatus*	5243 Da, 47aa	555 nM	4, 106
Elegantin	*Trimeserusus elegans*	7806 Da, 73 aa, pI 4.69, single chain	136 nM	100
Eristocophin	*Eristocophis macmahoni*			20
Eristostatin	*Eristocophis macmahoni*	5 400 Da, 49 aa	59 nM	110, 111
Flavoridin	*Trimeresurus flavoviridis*	70 aa	50 nM	7
Flavostatin	*Trimeresurus flavoviridis*	7 304 Da, 68 aa	59-111 nM	112, 113
Gabonin	*Bitis gabonica*	21 000 Da, 84 aa, disulfide-linked homodimer, pI 9.2	340– 1600 nM	114
Halysin	*Agkistrodon halys*	7500 Da, 71aa single chain	160-360 nM	115
Kistrin	*Calloselasma (Agkistrodon) rhodostoma*	7318 Da, 68aa	105-128 nM	106
Multisquamatin	*Echis multisquamatus*	5700 Da	97-333 nM for platelet of different species	116
Rhodostomin	*Calloselasma (Agkistrodon) rhodostoma*			7, 12
Salmosin 1 Salmosin 2 Salmosin 3	*Agkistrodon halys brevicandus*	7474 Da, 73 aa, single chain 73 aa (by cDNA) 80 aa (by cDNA)	131 nM	117, 118
Tergeminin	*Sistrurus c. tergeminus*			20
Triflavin	*Trimeresurus flavoviridis*			119, 120

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Trigramin Trigramin β_1 Trigramin β_2 Trigramin γ	*Trimeresurus gramineus*	7 500 Da, 72 aa, pI 5.61 7 551 Da, 72 aa 7 623 Da, 73 aa 7 563 Da, 73 aa	 300 nM 170 nM 240 nM	5, 106, 121
Trimucrin	*Trimeresurus mucrosquamatus*	71 aa (by cDNA)	¾	122
Ussuristatin 1 Ussuristatin 2	*Agkistrodon ussuriensis*	7 458 Da, 71 aa, single chain 7 385 Da, 71 aa, disulfide-linked homodimer	17-33 nM 140-290 nM	21
Venom inhibitor	*Calloselasma (Agkistrodon) rhodostoma*	31 000 Da, 266 aa dimer, glycoprotein, 8.3 % neutral sugars	160-320 nM	123

Table 2. Class I. Antagonists of fibrinogen receptor glycoprotein IIb/IIIa complex (GPIIb/IIIa) that are unrelated to disintegrins

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Mambin	*Dendroaspis jamesonii*	6 744 da, 59 aa	172 nM	47
Decorsin	*Macrobdella decora*	4379 Da, 39 aa, pI 4.45	500 nM	49
Ornatins A2	*Placobdella ornata*	4449.6 Da, 41 aa, pI 9.8	133 nM	50
A3		pI 9.8	¾	
B		5868.2 Da, 52 aa, pI 8.75	¾	
C		5839.5 Da, 52 aa, pI 9.45	279 nM	
D		5721.6 Da, 44 aa	¾	
E		5722.5 Da, 50 aa, pI 8.75	438 nM	
Variabilin	*Dermacentor variabilis*	4 969 Da, 47 aa	157 nM	51
Salivary gland extract of deerfly	*Chrysops*	¾	¾	124
Disagregin	*Ornithodoros moubata*	6 987 Da, 60 aa, pI 7.35	104 nM	52

Table 3. Class II. Antagonists of von Willebrand factor receptor glycoprotein Ib (GPIb)

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Agkicetin	*Agkistrodon acutus*	26 000 Da, disulfide-linked heterodimer	12.5 nM	125
CHH-A CHH-B	*Crotalus horridus horridus*	23 000 Da, disulfide-linked heterodimer 25 000 Da, disulfide-linked heterodimer (α= 15 000, β=12 000 Da)	¾	126
Echicetin	*Echis carinatus*	26 000 Da, disulfide-linked heterodimer, 16 000 , 14 000 Da, C-type lectin	5.77-42.3 nM	56, 57
Flavocetin-A Flavocetin-B	*Trimeresurus flavoviridis*	149 000 Da, subunits α=17 000 Da, β=14 000 Da 139 000 Da, subunits α=17 000,β=15 000 Da, γ=14 000 Da	1.0-3.3 nM 1.80-4.00 nM	55
Jararaca GPIb-BP	*Bothrops jararaca*	30 000 Da, disulfide-linked heterodimer, α= 142 aa (17 457 Da), β=123 aa (15,035 Da)	28-42 nM	127, 128
Tokaracetin	*Trimeresurus tokarensis*	28 900 Da, disulfide linked heterodimer (16 100, 15 400 Da)	8.65-20 nM	129

Table 4. Class III. Antagonists of collagen-platelet interaction

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Calin	*Hirudo medicinalis*	65 000 Da	¾	66
Leech antiplatelet protein (LAPP)	*Haementeria officinalis*	16 000 Da, 126 aa	60 nM	67, 68
Moubatin	*Ornithodoros moubata*	17 000 Da	50 nM	69, 70
Tick adhesion inhibitor (TAI)	*Ornithodoros moubata*	15 000 Da	¾	71
Pallidipin	*Triatoma pallidipennis*	19 000 Da	50-200 nM	72
Catrocollastatin-C	*Crotalus atrox*	23 600 Da	66 nM	59, 62
Crovidisin	*Crotalus viridis*	53 000 Da, single chain	0.17 μM	65
Jararhagin-C	*Bothrops jararaca*	28 000 Da, 212 aa	N. D.	61

Table 5. Class IV. Antagonists of thrombin-thrombin receptor interaction

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
Bothroalternin	*Bothrops alternatus*	27 000 Da, disulfide-linked homodimers (14 000 Da), C-type lectin	7 nM	76
Bothrojaracin	*Bothrops jararaca*	27 000 Da, pI 4.2, disulfide-linked heterodimer (15 000, 13 000 Da)	2.2 nM	75
Triabin	*Triatoma pallidipennis*	17 000 Da, 142 aa	2.6 nM	77-79

Table 6. Class V. Other nonenzymatic inhibitors which inhibit platelet aggregation through unknown mechanism

Name	**Species**	**Physical properties**	**IC_{50}**	**References**
Lebetin 1α Lebetin 1β Lebetin 2α Lebetin 2β	*Vipera lebetina*	1 306 Da, 13 aa 1 249 Da, 12 aa 3 944 Da, 38 aa 3 886 Da, 37 aa	27-125 nM 5-48 nM	80, 81

Table 7. Nucleotidases that inhibit platelet aggregation

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
5'-nucleotidase	*Trimeresurus gramineus*	74 000 Da, single glycoprotein chain, 589 aa, 22% neutral sugars, Hydrolysis: AMP: 15.8 µmol Pi/min per mg ADP: 3.2 µmol Pi/min per mg	0.68 µM	82
ADPase	*Agkistrodon acutus*	94 000 Da, 852 aa	¾	83
ADPase/5'-nucleotidase	*Vipera aspis*	Hydrolysis: AMP: 1.03 µmol Pi/min per mg ADP: 2.03 µmol Pi/min per mg	¾	84

Table 8. Phospholipase A_2 enzymes that inhibit platelet aggregation

Species	Name	Physical Properties	IC_{50}	References
Acanthophis antarcticus	Acanthin I Acanthin II	12 845 Da, 119 aa, pI 10.2 12 896 Da, 118 aa, pI 10.4	7 nM 4 nM	130
Acanthophis praelongus	Praelongin 2bIII Praelongin 2cII Praelongin 2cIV	12 783Da, pI 10.3 12 971 Da, pI 9.4 12 972 Da, pI 9.6	650 nM 180 µM 55 µM	131
Agkistrodon acutus	Phospholipase A_2	16 4000 Da, pI 4.9	¾	91
Agkistrodon halys	Phospholipase A_2	14 000 Da, 130 aa, single chain, <1% carbohydrate	0.78 µM	92, 132
Agkistrodon halys pallas	Phospholipase A_2	124 aa, pI 4.5 single chain	¾	133
Austrelaps superba	Phospholipase A_2	15 000 Da	0.33 µM	87, 134
Austrelaps superbus	Superbin I Superbin II	13 252 Da 13 235, 13212.9 Da	¾	135
Echis carinatus	EC-I-PLA_2	16 000 Da	~ 2 µM	136
Lachesis muta	LM-PLA_2	17 000 Da, pI 4.7, single chain	25-125 nM	137
Naja naja atra	phospholipase A_2	¾	¾	86
Naja nigricollis	CM-I CM-II CM-IV	15 000 Da 15 000 Da 15 000 Da	¾	88
Ophiophagus hannah	OHV A-PLA_2	13 719 Da, 124 aa, single chain	4-1530 nM	89, 138
Pseudechis papuanus	PPV	15 000 Da	¾	90
Trimeresurus graminues	Platelet aggregation inhibitor	12 400 Da, 109aa, pI 3.6, single chain	0.2-0.4 µM	139, 140
Vipera russelli	VRV-PL-IIIb	14-15 000 Da, pI 7.3-7.7	¾	141
Vipera russelli formosensis	Phospholipase A_2	¾	¾	142
Vipera russelli siamensis	Venom inhibitor	13 800, 123 aa, pI 10.4	26.8-82.6 µM	143, 144
Apis mellifera	Phospholipase A_2	¾	¾	145
Heloderma horridum	HHV-PLA	19 000 Da, 163 aa, single chain	¾	146

Table 9. Metalloproteinases and their related domains that inhibit platelet aggregation

Name	**Species**	**Physical Properties**	**IC_{50}**	**References**
α-Fibrogenase	*Calloselasma rhodostoma*	25 400 Da, 226 aa, single chain, pI >10, <1% carbohydrate	¾	147, 148
α-Fibrogenase	*Trimeresurus mucrosquamatus*	22 400 Da, 203 aa, pI 8.1, 2% carbohydrate	¾	95, 149, 150
Fibrogenase	*Vipera lebetina*	26 000 Da, pI 5.9, glycoprotein, 5% carbohydrate	¾	151, 152
Jararhagin	*Bothrops jararaca*	52 000 Da	40 nM	63, 64, 153
Kistomin	*Calloselasma rhodostoma*	21 800 Da, 202 aa, single chain	0.37 μM	97, 154
Protease L4	*Agkistrodon halys brevicaudus*	22 000 Da, 173 aa	¾	155
Proteinase F1	*Naja nigricollis*	58 000 Da, pI >10, single chain	2μM	96, 156
Atrolysin A and Atrolysin A/DC	*Crotalus atrox*	24 479 Da, probably glycosylated	320-470 nM	60
Atrolysin E/D	*Crotalus atrox*	7 400 Da, 68 aa	4-8 nM	98
Catrocollastatin	*Crotalus atrox*			157