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like proteins (CLPs or snaclecs) [7–9]. These non-

$_i$ = 0.52 mole) in a Ca^{2+}-independent manner; however, supplementation with 0.25 mM Ca^{2+} enhanced the Xa binding potency of RVsnaclec. Monovalent or polyvalent antivenom failed to neutralize its anticoagulant potency, and RVsnaclec did not inhibit trypsin, chymotrypsin, thrombin or plasmin. RVsnaclec was devoid of hemolytic activity or cytotoxicity against several human cancer cell lines, demonstrated concentration-dependent aggregation and deaggregation of human platelets, and inhibited the ADP-induced aggregation of platelet. RVsnaclec (5.0 mg/kg body weight) was non-lethal to mice and showed no adverse pharmacological effects, suggesti[26].Venoms from many species of snakes, particularly those of the family Viperidae, contain moderate to high amounts of C-type lectin-

$^{2+}$ -

dependent proteins derive their name from the sequence homology (15–40%) with the carbohydrate recognition domains of C-type lectins [7,8]. The basic structure of snaclecs is often composed of - heterodimers which are covalently linked by a disulfide bridge; however, these heterodimers may also form non-covalent multimeric forms, thus giving rise to , ( )$_2$, and ( )$_4$ structures [8]. The subunits of these heterodimers typically show significant sequence identify [7,8]. Despite their structural similarity, the snaclecs have exceptionally diverse biological functions, targeting coagulation factors, membrane receptors and platelets receptors. A major effect of these venom components upon envenomation is the disruption of hemostatic mechanisms of the victim/prey [7,8].

The anticoagulant mechanism of snaclecs involves binding with high affinity to specific blood coagulation factors such as IX and/or X [10,11]. To date, several IX/X-binding snaclecs have been purified from the venoms of numerous vipers, including Trimeresurus flavoviridis, Bothrops jararaca, Agkistrodon halys pallas, Deinagkistrodon acutus, and Echis carinatus leucogaster[for review see 7–9]. However, the

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$$Km_{App} = Km(1 + I/Ki) \quad (2)$$

$$Y = Vmax_{App} X/(Km_{App} + X) \quad (3)$$

where the constant I is inhibitor concentration, $Vmax_{App}$ and Vmax are maximum velocity in the presence and absence of the inhibitor (RVsnaclec), respectively, Km_{App} and Km are the Michaelis constant in presence and absence of inhibitor, respectively, and is a constant.

Spectro uorometric analysis of interaction of RVsnaclec with FXa

To study protein-protein interactions, 0.1 g FXa was pre-incubated with 1.0 g Ca^{2+} -free RVsnaclec (1:10 ratio) in the absence or presence of 0.25 mM Ca^{2+} for 10 min at room temperature. The uorescence

the range of 14‥15 and 13‥14 kDa, respectively [7,8,16]. Therefore, the molecular mass of subunit of RVsnaclec is typical of the size of the larger subunit of snaclecs, but the molecular mass of the subunit is the smallest reported to date among snaclecs. From the molecular mass of native RVsnaclec as determined by MALDI-TOF-MS, native RVsnaclec apparently consists of ($)_2$-($)_4$. Based on a mean residual molecular mass of 113 Da per amino acid, the subunit of RVsnaclec consists of approximately 131-132 amino acid residues, which is in accordance with the amino acid residue number present in other snaclecs [8,16]. However, the subunit of RVsnaclec likely consists of only 88-89 amino acid residues, far lower than the smaller subunit of other snaclecs.

LC-MS/MS identi cation of RVsnaclec

The LC-MS/MS analysis of RVsnaclec showed signi cant matching with Snaclecs puri ed from Viperidae venom such as C-type lectin-like 3 (accession no. 7362011, -10lgP: 307.33, sequence converge 78%, unique peptide 35), C-type lectin-like protein subunit 3 from *Daboia siamensis* (accession no. 67043477, -10lgP: 307.33, sequence coverage 78%, unique peptide 35), and C-type lectin from *Macrovipera lebetina* (accession no. 158906219, -10lgP: 258.60, sequence coverage 53%, unique peptide 2). The BLASTP analysis of tryptic peptide sequences K.GSHLLSLHNIAEADFVLK.K (m/z 982.5356), and M.GLNDVWNEC(+57.02) NWGWTDGAK.L (m/z 1061.459) of RVsnaclec demonstrated putative conserved domains of C-type lectins (CTL) or the carbohydrate-recognition domain (CRD), a typical feature of snaclecs. Taken together, RVsnaclec is the rst example of a snaclec from *D. r. russelii*, and it represents a new C-type lectin-like protein from snake venom.

Biochemical properties, anticoagulant activity and inhibition of serine proteases

RVsnaclecs did not demonstrate PLA_2, metalloprotease, brinolytic, brinogenolytic, BAEE or TAME-esterase activities. RVsnaclec showed potent anticoagulant activity which increased linearly with increasing concentration, from 0.75 to 3.0 g/ml (Fig. 2A). However, above 3.0 g/ml, RVsnaclec did not show an increase in the re-calci cation time of PPP (Fig. 2A). With an increase in the pre-incubation of PPP/RVsnaclec from 1-10 min, a concomitant increase in anticoagulant activity of RVsnaclec was observed, but after 10 min of pre-incubation with PPP, the anticoagulant activity of RVsnaclec was not enhanced further (data not shown). This result indicates that, similar to Rusvikunin, a Kunitz-type anticoagulant peptide puri ed from RVV [6], binding of RVsnaclec with FXa is a very rapid event. The anticoagulant activity of RVsnaclec appears quite stable and remained unaffected after 5 cycles of freeze-thawing (data not shown).

RVsnaclec dose-dependently increased the prothrombin time of platelet-(30)etn3(na)25.7(9348)Fig. 2B), but it (n3(na)2did)-258.6(no)13.7(t)-269.1(a)0(f)

Based on their pharmacological targets, the anticoagulant snaclecs can be classi ed into three speci c categories [16]. Snaclecs belonging to class 1 are coagulation factors IX/X-binding proteins. The class 2 and 3 snaclecs bind only to coagulation factor IX and coagulation factor X, respectively [16]. Owing to its binding capacity with FX, RVsnaclec belongs to class 3 snaclecs. In the presence of Ca^{2+}, these proteins have been shown to bind to the -carboxyglutamic acid (Gla) domain of coagulation factor X [8,10,16]. However, RVsnaclec also signi cantly

this may also be the role of Ca^{2+} for recognition followed by FXa inhibition by RVsnaclec.

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