Supplementary Information

Supplementary Figures

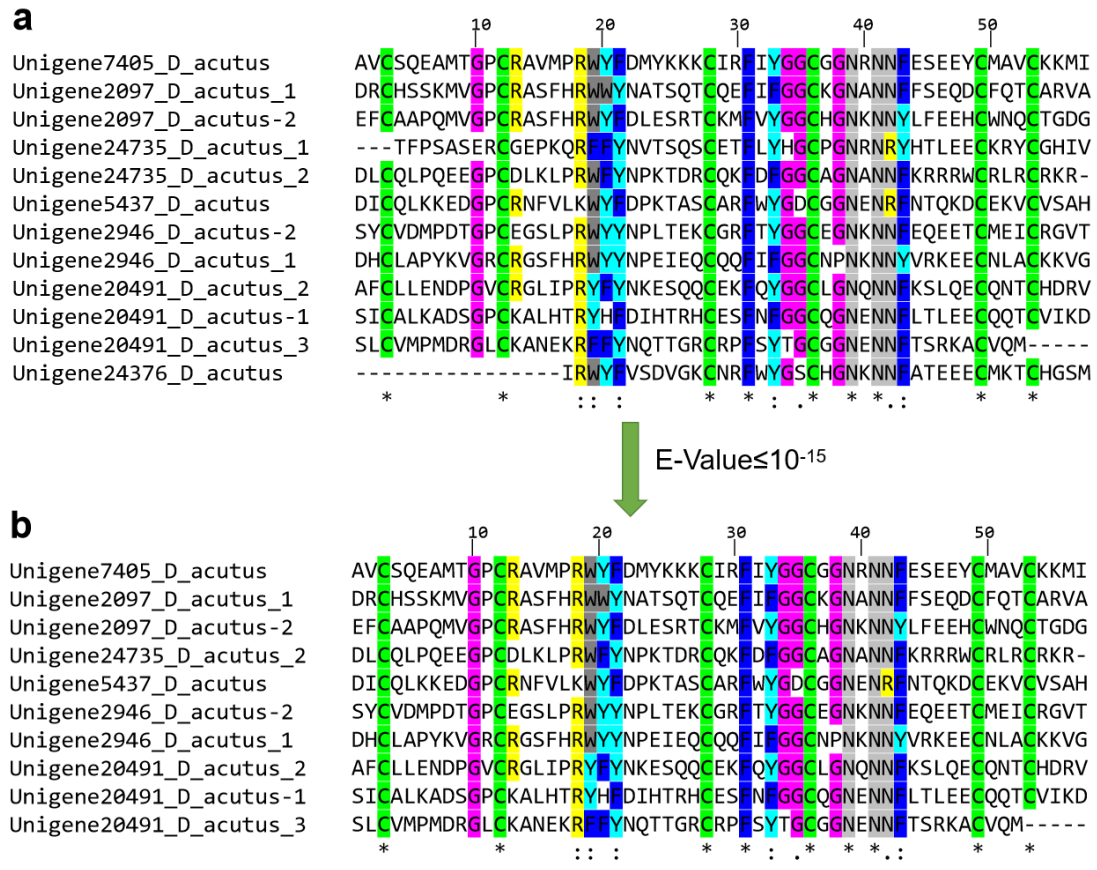

Figure S1. Alignment of amino acid sequence of Kunitz domains from venom of *D. acutus*. **(a)** The multiple sequence alignments of 12 Kunitz scaffold-based peptides recognized by the Pfam database. **(b)** The multiple sequence alignments of 10 Kunitz scaffold-based peptides recognized by the Pfam database when the E-Value is below 10^{-15}. "*" indicates positions with fully conserved residue. ":" indicates conservation between sequences with similar properties and "." indicates conservation between groups of weak similar properties.

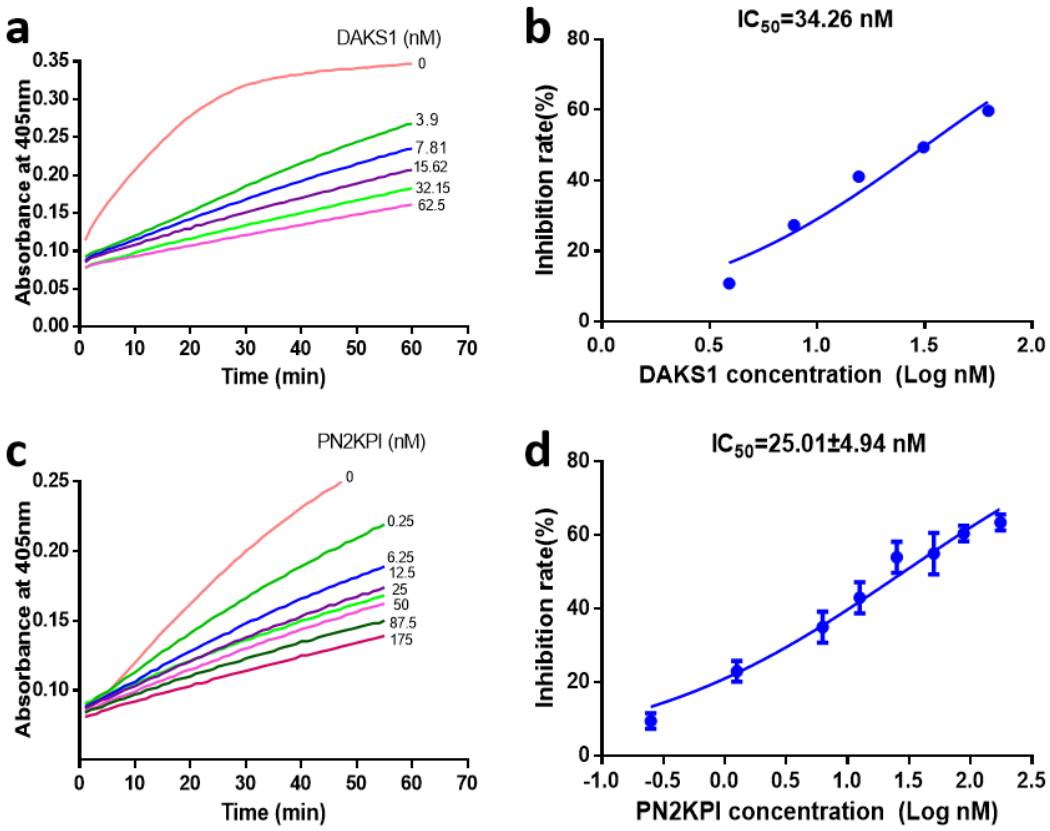

Figure S2. DAKS1 inhibited Trypsin. **(a)** The Inhibition activity of DAKS1 towards Trypsin. Reactions started by addition of CS-11(22) (250 μM) to a mixture containing DAKS1 (0-125 nM) incubated for 60 mins with Trypsin (4.15 nM). Substrate was hydrolyzed monitored at 405 nm for 60 mins. (**b)** Determination of the IC_{50} of DAKS1. Plot of inhibition rate (%) vs log DAKS1 concentrations was fitted by nonlinear regression and the value of IC_{50} was obtained by fit log(inhibitor) vs. normalized response-variable slope. (**c)** The Inhibition activity of PN2KPI towards Trypsin. Reactions started by addition of CS-11(22) (250 μM) to a mixture containing PN2KPI (0-175 nM) incubated for 60 mins with Trypsin (4.15 nM). Substrate was hydrolyzed monitored at 405 nm for 60 mins. (**d)** Determination of the IC_{50} of PN2KPI. Plot of inhibition rate (%) vs log PN2KPI concentrations was fitted by nonlinear regression and the value of IC_{50} was obtained by fit log(inhibitor) vs. normalized response-variable slope. Data are presented as the mean ± SD of three independent experiments.

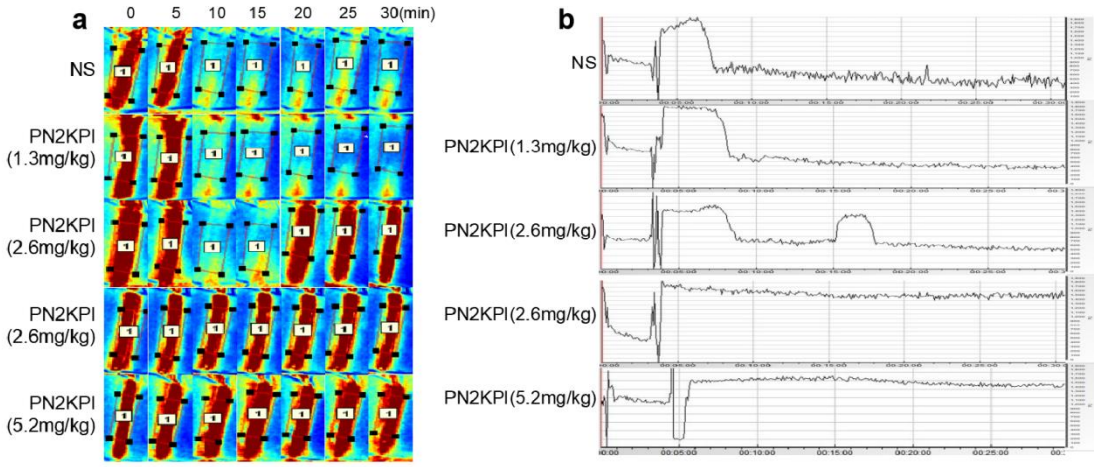

Figure S3. PN2KPI inhibited $FeCl_3$-induced arterial thrombosis formation. (**a)** Saline and PN2KPI(1.3, 2.6 and 5.2mg/kg) were injected through a tail vein 10 min before $FeCl_3$-induce, and blood flow at region of interest (ROI) was monitored at 0, 5, 10, 15, 20, 25 and 30 min. (**b)** Representative blood perfusion graphs of mice treated with ,NS, PN2KPI. NS: Normal Saline.