Allosterism-based Simultaneous, Dual Anticoagulant and Antiplatelet Action. Allosteric Inhibitor Targeting the GPIbα and Heparin-Binding Site of Thrombin

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Methods

Fibrinogen Assay

SbO4L was tested against human thrombin's ability to cleave its physiological substrate human fibrinogen using a microplate assay. Briefly, in 35 μL of buffer (20mM TrisHCl, 100mM NaCl, 2.5mM $CaCl_2$ 0.1% PEG8000, pH 7.4) was added 10 μL of thrombin (300nM) and 5 μL of either water as control or SbO4L at various concentrations. The mixture was incubated for 10 minutes, after which 10 μL of this reaction was added to 140 μL of 250 nM fibrinogen (Haematologic Technologies (Essex Junction, VT)). The final concentration of thrombin was hence 4 nM. The formation of fibrin mesh was observed as absorbance at 600 nm for the first 100 seconds after initiation of the reaction. The slope was used to get the rate of enzyme activity towards fibrin formation activity. The experiment was done in duplicate. The percent residual activity and IC_{50} were calculated in a manner similar to that done for the peptide substrate reactions.

Figures

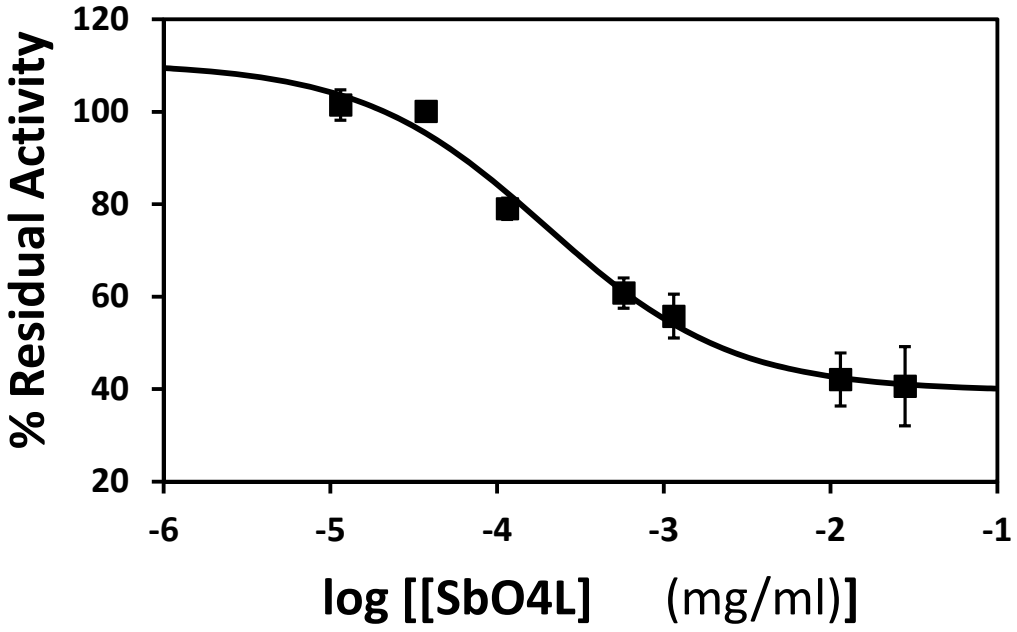

Figure S1. Direct inhibition of human thrombin by SbO4L using Fibrinogen as substrate. The residual activity of thrombin at pH 7.4 and 37 °C was measured using physiologic substrate fibrinogen in a buffered microplate assay. The sigmoidal dose-response curve was used to obtain an IC_{50} of 0.19 ± 0.09 μg/mL.

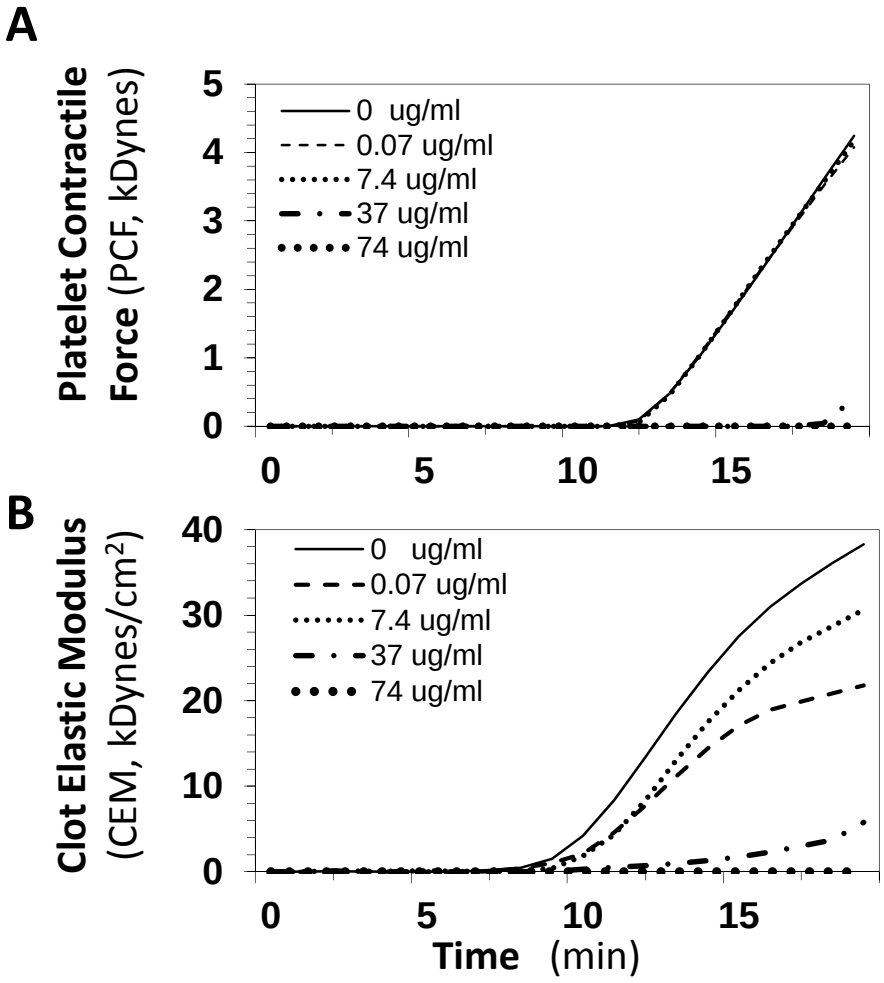

Figure S2. The effect of SbO4L on platelet function in human PRP using hemostasis analysis system (HAS^{TM}). (A) and (B) show the change in platelet contractile force (PCF) and clot elastic modulus (CEM), respectively, with time at varying concentrations of SbO4L (0 to 74 μg/ml) using PRP (platelet count adjusted to ~200,000).