High Throughput Screening Assay to Identify Small Molecule Inhibitors of Gα12 / α-SNAP-dependent vWF Secretion

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BACKGROUND

- Inflammatory states such as cancer, Type-2 Diabetes and severe infections lead to cardiovascular complications with greater risk of developing life-threatening blood clots.
- These conditions are associated with elevated blood pressure and pro-inflammatory cytokine levels leading to endothelial cell activation and secretion of von Willebrand Factor (vWF), a major factor in both hemostatic and thrombotic states causing platelet adhesion and subsequent thrombus formation (Ref. 1).
- Previous findings demonstrated Gα12 interacts with α-SNAP (soluble N-ethylmaleimide sensitive factor attachment protein) and is required for α-SNAP dependent vWF secretion (Ref. 2).
- Further studies in the Minshall lab identified a unique 6 amino acid sequence from the Gα12 amino terminal domain essential for α-SNAP binding (SBD6).
- A cell permeable myristoylated SBD6 is a peptide antagonist of Gα12/α-SNAP interaction that blocks both basal and thrombin induced vWF secretion in human endothelial cell cultures and reduces thrombosis in an animal model of sepsis (Figure 1, Ref. 3)

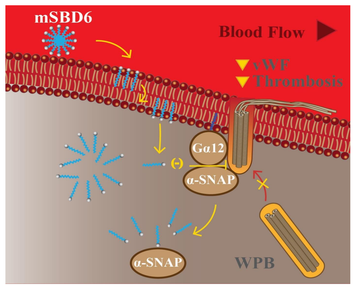


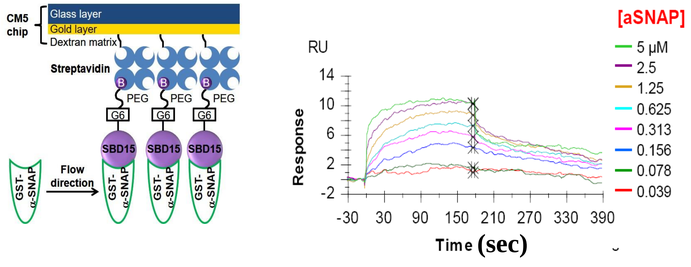
Figure 1

OBJECTIVE

- Design, develop and execute HTS to identify small molecule mimetics of SBD6 to ultimately inhibit vWF secretion by blocking the Gα12/α-SNAP interaction.

METHODS

- Surface plasmon resonance (SPR) studies show that SBD6 but not SBD6 scrambled peptide bound directly to immobilized GST-αSNAP with K_D= 12.9 μM (Ref 4)
- SBD6 was re-designed to include additional Gα12 sequence, a G6 and PEG linker and biotin tag at the C-terminus (SBD15-G6-PEG-Biotin, SBD-Biotin)
- SPR with SBD-Biotin showed improved binding affinity for GST-αSNAP (K_D= 0.37 μM), suitable for use in High Throughput Screen (HTS) assay **(Figure 2)**



Peptide	K_D	R_{max} (%)
SBD15-Biotin	0.37 μM	11.6

Figure 2, Ref 4

- AlphaLISA was chosen for HTS assay development.
- Chemical energy transfer from donor to acceptor bead is based on proximity through the biological interaction of the peptide and protein. **(Figure 3A)**
- Presence of inhibitor increases distance from donor to acceptor bead and Alpha signal is decreased **(Figure 3B)**
- GST-αSNAP and SBD-Biotin titration using streptavidin donor beads paired with glutathione acceptor bead generated a suitable AlphaLISA signal for use in HTS **(Figure 3C)**
- Unlabeled SBD15 blocked the SBD-Biotin GST-αSNAP, demonstrating acceptable statistical window for HTS and K_D in agreement with SPR result **(Figure 3D)**

Amplified Luminescent Proximity Homogeneous Assay-Linked ImmunoSorbent Assay (**AlphaLISA**)

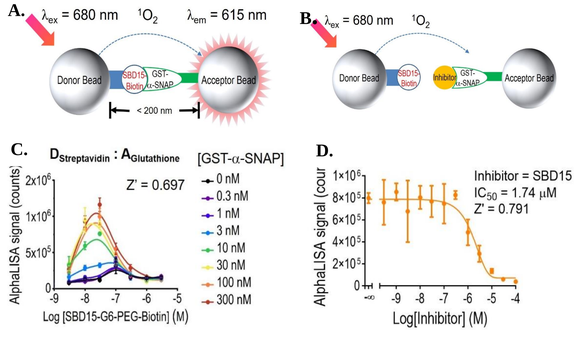


Figure 3, Ref 4

HTS

- The αSNAP-SBD-Biotin ALphaLISA was used to screen a 10,000 chemically diverse small molecule library from ChemDiv **(Figure 4 Example of 1 plate)**
- Assay performance was robust with average Z' of 0.78 (**Figure 5)**
- Overall hit rate a little high but manageable **(Figure 6)**

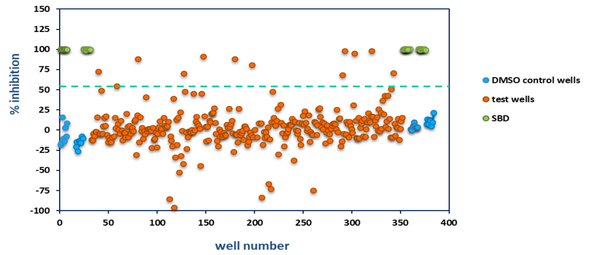


Figure 4

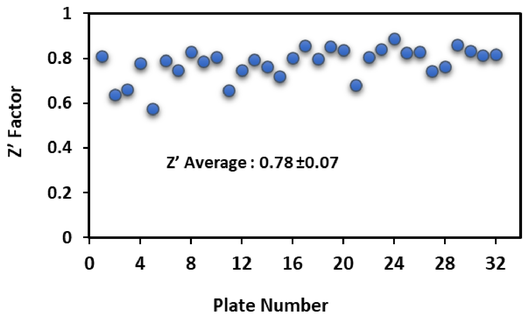


Figure 5

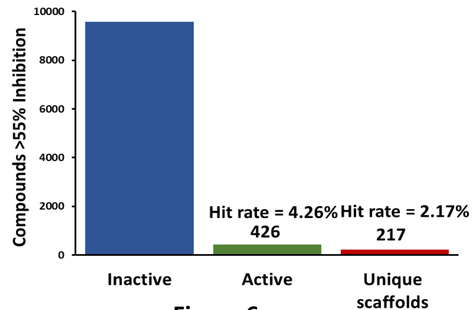


Figure 6

- Compound triage has been completed through this series of assays (Figure 7)
- 4 compounds demonstrated shift ranging from 0.92-4.0 °C in the αSNAP thermal shift assay (Figure 7)
- Binding of the 4 compounds to αSNAP will be confirmed by a second biophysical method (SPR, Figure 8) and then advanced to cellular assays
- Scaffolds with the following **Target Profile** will be advanced
  - IC50 <20 μM αSNAP-SBD AlphaLISA
  - Confirmed binding to αSNAP by thermal shift and SPR
  - Demonstrated activity in EC vWF ELISA and IP at 20 μM

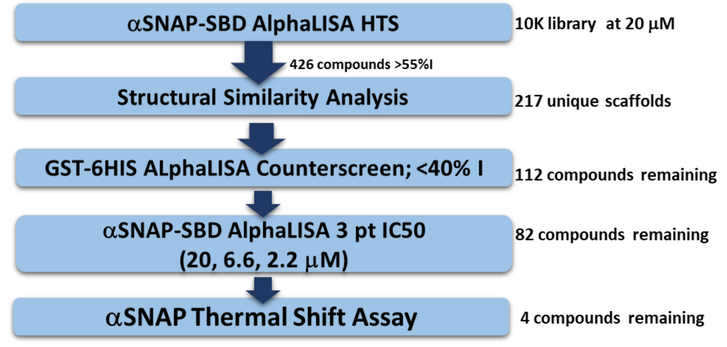


Figure 7: Compound Triage Complete

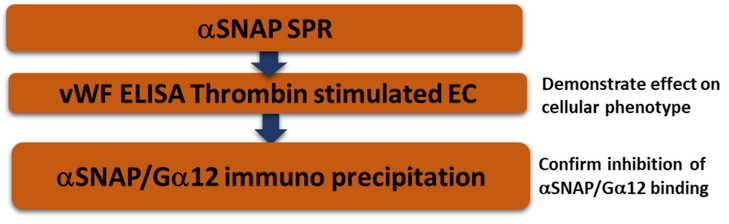


Figure 8: Compound Triage in Process

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