Activating Challenging GPCR Targets with Venom Peptides

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Abstract

Peptides found in snake and spider venom can activate GPCRs, highlighting the potential of natural products as a novel source of tool compounds or hit-to-lead chemistry in drug discovery.

Project Aim: To use venom peptides as a novel approach to activate difficult GPCR targets using high throughput techniques.

Introduction

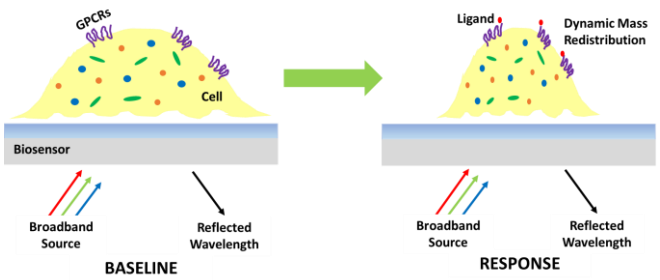
- G-protein coupled receptors (GPCRs) are a superfamily of transmembrane signalling proteins. They represent more than 50% of drug targets in the clinic (Lundstrom, 2006)
- The pharmaceutical industry faces a problem of a narrowing pipeline in GPCR ligand discovery.
- Venom peptides, like the three-finger toxins found in snake venom (Rajagopalan et al, 2007) and latrotoxins found in spider venom (Davletov et al, 1996), have been found to activate GPCRs.
- Previous attempts to identify agonists / antagonists of GPR120, GPR39, VIPR1 and PAR2 via High Throughput Screening (HTS) had failed to yield promising compounds.
- A venom peptide library, specifically targeting GPCRs, was profiled against the selected receptors.

Venom Peptide Library

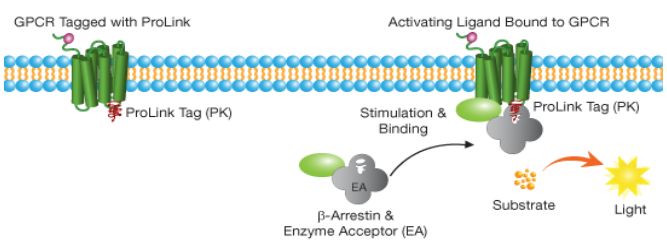
- T-VDA GPCR library containing lyophilized venom fractions provided by Venomtech.
- Solubilised in PBS and screened at nominal 100 nM final concentration.

High Throughput Assays

Corning Label-Free Detection Epic Technology
Detects changes in cellular dynamic mass redistribution (DMR).



Discover-X PathHunter® β-Arrestin Assay
Measures β-gal activity using PathHunter® chemiluminescent Detection Reagents



Courtesy of DiscoverX

Results

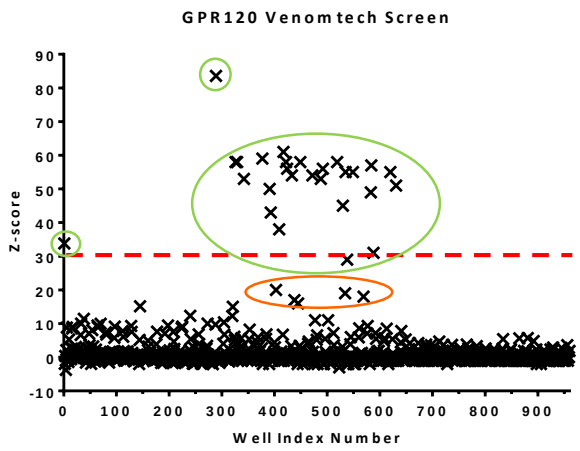


Figure 1. ***GPR120 Venomtech Screen***
Venomtech's T-VDA GPCR library screened against GPR120 in a DMR assay. Wells with a Z-score> 30 identified as containing a venom fraction eliciting an agonist effect.

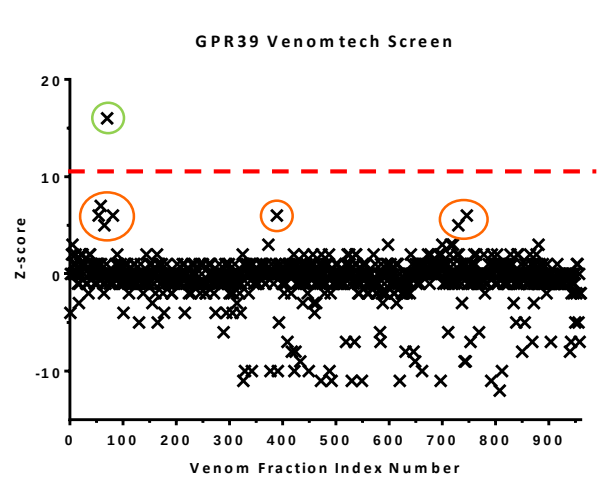


Figure 2. ***GPR39 Venomtech Screen***
Venomtech's T-VDA GPCR library was screened against GPR39 in a DMR assay. Wells with a Z-score> 10 identified as containing a venom fraction eliciting an agonist effect.

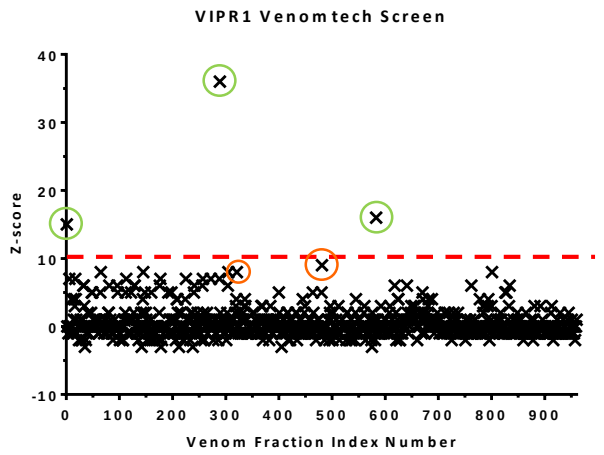


Figure 3. VIPR1 Venomtech Screen
Venomtech's T-VDA GPCR library was screened against VIPR1 in a DMR assay. Wells with a Z-score> 10 identified as containing a venom fraction eliciting an agonist effect.

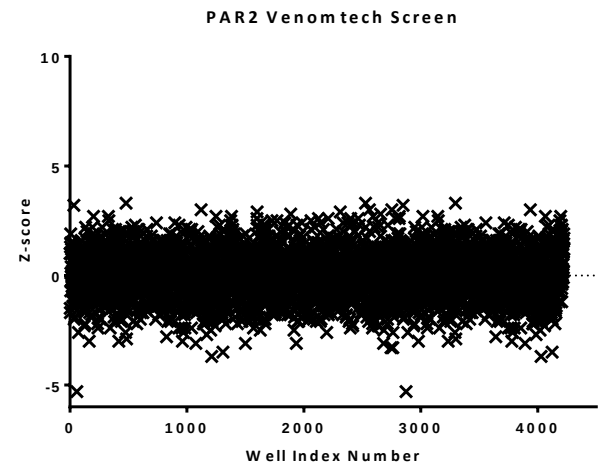


Figure 4. PAR2 Venomtech Screen
Venomtech's T-VDA GPCR library was screened against PAR2 in a β-arrestin antagonist assay. No wells identified as containing a venom fraction that had an antagonist effect on PAR2.

Table 1. ***Venomtech Screen Summary***
The venom species that had fractions identified as displaying **activity**, **weak activity** and **no activity** against each GPCR in the screen.

Venom Species	GPR120	GPR39	VIPR1	PAR2
Dendroaspis angusticeps	●	●	●	X
Dendroaspis polylepis	X	● ●	X	X
Dendroaspis viridis	X	●	X	X
Naja kaouthia	● ●	X	●	X
Naja sputatrix	● ●	●	X	X
Naja siamensis	● ●	X	●	X
Bothrops atrox	●	X	X	X
Crotalus atrox	X	X	X	X
Crotalus ruber	X	X	X	X
Hysterocrates gigas	X	X	X	X
Chilobrachys guangxiensis	X	●	X	X
Poecilotheria regalis	X	X	X	X

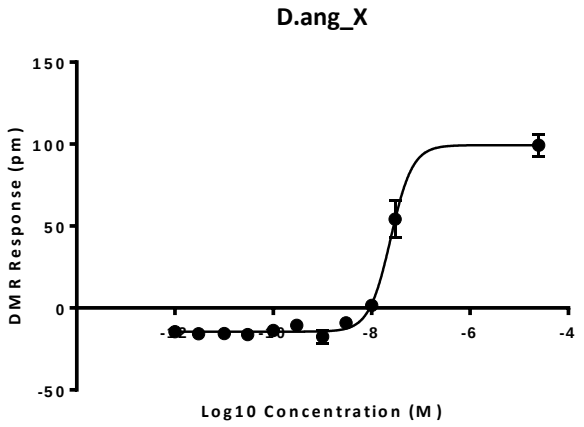


Figure 2. ***D.ang_X Activity Against GPR120.***
A fraction belonging to *Dendroaspis angusticeps* was tested against GPR120 in a dose response from a top nominal final concentration of 30 nM with half log dilution steps.
EC_{50}= 26 nM.
Results presented as mean ± S.D. (n=3).

Conclusions

- Initial hits identified against GPR120, GPR39 and VIPR1. No venom fractions were found to have antagonist activity against PAR2.
- Potential weaker additional hits against the three targets may also have been identified
- The D.ang_X fraction confirmed to activate GPR120 in a dose dependent manner.
- Retesting and further work to confirm and characterise the hits reported here is ongoing.

References

1. Latest development in drug discovery on G protein coupled receptors, K, Lundstrom, Curr Protein Pept Sci 2006
2. Beta-cardiotoxin: a new three-finger toxin from Ophiophagus hannah (king cobra) venom with beta-blocker activity, N Rajagopalan et al, Faseb J 2007
3. Isolation and biochemical characterization of a Ca2+-independent α-latrotoxin-binding protein, B. A Davleotov et al, J. Biol. Chem 1996.

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