Bridging the gap between venom research and modern drug discovery

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1 Abstract Summary

❖ Where are the next venom derived drugs coming from?

❖Good assays are only the starting point. These can then be used to assess activity in High Throughput Screening (HTS) to identify hits.

❖Most patient populations prefer a single daily oral tablet.

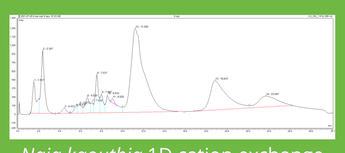
❖The challenge for all drugs is how does the drug get to the site of action at high enough concentration to be efficacious and yet avoid side effects. These are also challenges for venom drug discovery and this poster presents this experimental journey illustrated with data

2 Venom Preparation

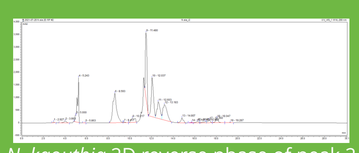
❖Venoms are complex mixtures – assays require simplicity for high throughput and accurate data interpretation.

❖Venomtech use in-line 2D ultra High Performance Liquid Chromatography (uHPLC) where the 1st dimension fractions are directly injected into the 2nd dimension without intermediate processing.

❖Fractions are formatted in Echo® qualified 384 well plates for HTS dispensing called Targeted- Venom Discovery Arrays (T-VDA™).



Naja kaouthia 1D cation exchange



N. kaouthia 2D reverse phase of peak 2

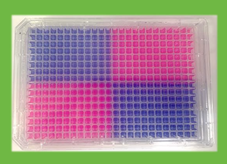
Echo® qualified 384 well plate

3 Assays

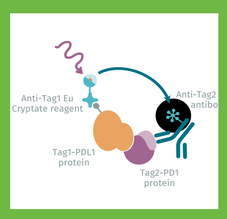
❖The fundamental 384-well plate, developed in 1992, is still the mainstay of pharmaceutical HTS platforms with 1536-well plates now growing in use.

❖Any assay system useful for understanding the action of a drug on a target is amenable to venom research. However, to be used in HTS they need to pass the Z' assay at >0.5 and <1.

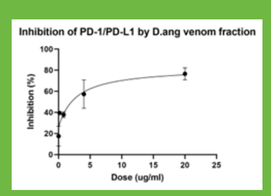
❖In 2021 Venomtech presented data of venom peptides being use in High Throughput Time Resolved Fluorescence (HTRF) an HTS validated assay.



HTS requires robust assays that repeat 1000's of times. The Z' assay tests this repeatability by using 3 full plates ½ positive control and ½ negative controls in a quadrant pattern. The signal window and variability measure calculated must be >0.5 and <1. our PD-1 assay produced Z' = 0.78.



PD1/PDL1 HTRF assay kit (cisbio) uses proximity assay to determine binding of PDL1 to PD-1. T-VDA dissolved in assay buffer, 2µl aliquoted into 384-well low volume proxiplate (Perkin Elmer). Plates read at 25°C in the CLARIOstarplus plate reader (BMG Labtech) using the TRF filter set with autofocus and dynamic range optimisation.



Hit follow up typically uses dose response curves to confirm hit pharmacology as being from a single active (first order curve) and of the desired potency. This assay delivered 22 hits from 640 venom fractions. The best were identified by intact mass and peptide digest MS/MS. The hits included Fasciculin from *Dendroaspis angusticeps*

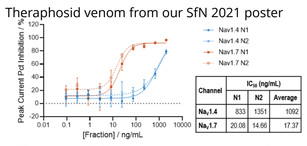
4 Hit to Lead Development

❖ Venoms proteins and peptides have many great properties that make them suitable for drug discovery – they have evolved to be stable, excellent potency and selectivity to targets and serendipitous actions useful for treating disease.

❖However, they have all evolved to be injected as this underpins the definition of venom. Even though some patient groups tolerate injections when there is no alternative, most prefer oral tablets.

The journey from Hit to Lead

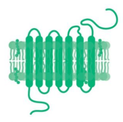
Theraphosid venom from our SfN 2021 poster



Channel	IC_{50} (ng/mL)		
	N1	N2	Average
$Na_V1.4$	833	1351	1092
$Na_V1.7$	20.08	14.66	17.37

Find the Hits that have required potency and selectivity

Drug like molecule
- Amenable to bulk production
- Small and stable
- Improvable properties
- Reduced off target effects



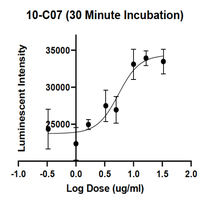
Understand the target
- Cellular location
- Tissue expression
- Compartment
- Disease relevant factors

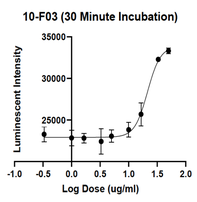
PK/PD and ADME
- Survives entry into patient
- Can engage target
- Stays on target long enough to cause therapeutic effect
- Slow clearance
- No toxic metabolites

PK/PD = Pharmacokinetics/Pharmacodynamics. ADME = Absorption Distribution Excretion and Metabolism

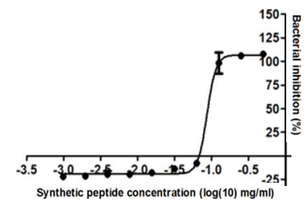
Turning venoms into drug like molecules

Venom proteins are often complex with post translational modifications making them challenging to synthesis or purify in large enough quantities to treat a population, so something smaller is needed.





We identified 18 hits from an agonist screen of GPR120 all with related sequences. Through Structure-Activity Relationship (SAR) studies we identified an active site of 14 key amino acids from 60.

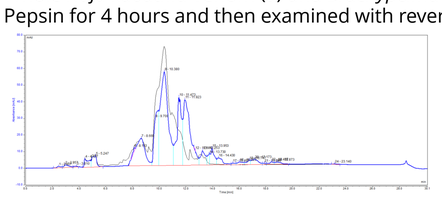


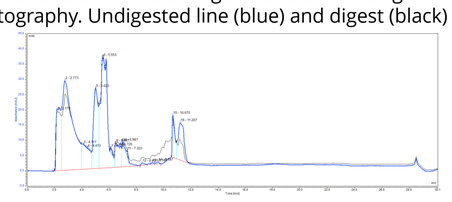
Inhibition of *Bacillus subtilis* growth with a 10 amino acid synthetic peptide from SAR investigation of scorpion venom peptides.

What do we know about venom ADME properties?

Venom proteins do have some promising signs of gastric stability and oral absorption potential. We have determined the oral toxicity of whole *Naja nigricollis* venom in rats as part of our operator safety work (Contracted to Charles River Laboratories). Results – *Naja nigricollis* oral LD50 between 50-300mg/kg using the fixed dose method.

Whole *Naja kaouthia* venom (a) and *Brachypelma boehmei* (b) were incubated in simulated gastric fluid with 3.2g/L Pepsin for 4 hours and then examined with reverse phase chromatography. Undigested line (blue) and digest (black)





5 Conclusions

❖Venoms contain hit molecules and when fractionated and/or purified produce robust target engagement at the molecular level *in-vitro*

❖Presented here are drug like interactions between venom peptides and diverse target classes including; Protein:Protein interactions, Voltage gated Sodium Channels and G-Protein Coupled Receptors

❖SAR can reduce complex venom proteins into peptides amenable to solid state synthesis and further optimisation

❖We are just starting to uncover the potential of oral peptide delivery and venom peptides are well positioned to lead the field in the further research needed.

12 YEARS ANNIVERSARY

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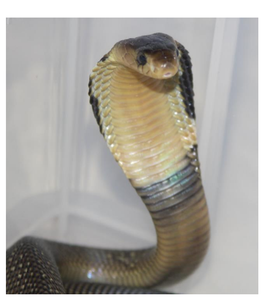

Acknowledgements
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