Identification of novel scorpion venom peptide inhibitors of the K_V1.3 ion channel and their potential as drug discovery leads for human T-cell mediated disease

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Introduction

Activated effector memory T-cells (T_{EM}) have been implicated in the pathogenesis of autoimmune diseases.[1] Activated T_{EM} cells express high levels of the voltage-gated potassium channel K_V1.3, which functions to control cell excitability. Inhibition of K_V1.3 reduces the release of pro-inflammatory mediators, inhibits T-cell proliferation and migration to inflamed tissues, and has been shown to ameliorate autoimmune disease symptoms in preclinical animal models. However, small molecule K_V1.3 inhibitors have failed to deliver a successful drug into the clinic, in part due to lack of potency and selectivity.

The evolutionary arms race between venomous animals and their prey has generated a diverse array of toxin peptides, many of which modulate ion channels. Toxin peptides are attractive starting points for drug discovery as they can offer improved potency and selectivity, which can be further improved alongside other drug-like properties such as pharmacokinetics by peptide engineering approaches.[3] One such example is ShK-186 (Dalazatide),[2] an optimised analogue of the native *Stichodactyla helianthus* (ShK) sea anemone neurotoxin originally identified as a potent but poorly selective K_V1.3 channel inhibitor.

ShK-186 is the first in-class K_V1.3 channel inhibitor to show clinical safety and efficacy, based on a Phase 1b psoriatic arthritis trial run by Kineta.[4] However, most K_V1.3 toxins, including ShK, are only moderately selective over other K_V1.x and K_{Ca} channel family members known to be expressed in T-cells.

Using Venomtech's proprietary Targeted-Venom Discovery Array platform (T-VDA™) and Metrion's high quality patch clamp electrophysiology assays, we collaborated to identify novel, potent and selective peptide toxin inhibitors of the human K_V1.3 channel.

Materials and Methods

A total of 370 venom fractions (5 – 10 peptides) from a variety of spider, scorpion and snake families (Table 1) represented by the Venomtech's T-VDA™ library were prepared by HPLC.

Group	Family	Genus	# samples
Spider	**Theraphosidae**	*Thrixopelma*	37
		Thrixopelma	36
		Poecilotheria	80
		Pterinochilus	30
		Cyriopagopus	78
		Hysterocrates	86
Scorpion	**Scorpionidae**	*Scorpio*	3
	Buthidae	*Parabuthus*	3
	Buthidae	*Centruroides*	3
	Scorpionidae	*Pandinus*	2
	Theraphosidae	*Monocentropus*	2
	Buthidae	*Androctonus*	2
	Caraboctonidae	*Hadrurus*	2
	Hemiscorpiidae	*Hadogenes*	2
Snake	**Elapidae**	*Naja*	2
		Dendroaspis	2

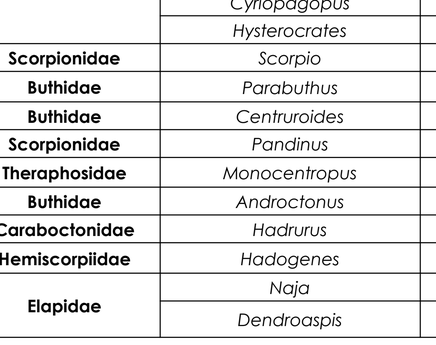
Table 1: Source of crude venom fractions used for the primary screening

The inhibitory effect of venom samples were tested against human K_V1.3 channels stably expressed in Chinese hamster ovary cells, using the QPatchHT gigaseal quality automated patch clamp system and standard solutions and voltage protocols (Figure 1). Venom samples lyophilized in sucrose (5 µM) were reconstituted in water before subsequent serial dilution in extracellular recording solution to achieve the final on-cell test concentrations. Fractions were tested in the presence of the carrier BSA (0.1%) to minimize non-specific binding. ShK (50 pM) and Sucrose (5 µM) were used as positive and negative controls, respectively.

A.

	Intracellular Solution (mM)	Extracellular Solution (mM)
NaCl	-	150
KCl	20	10
KF	120	-
HEPES	10	10
$MgCl_2$	-	1
$CaCl_2$	-	3
Glucose	-	10
EGTA	10	-
NaATP	5	-
pH	7.2 (KOH)	7.4 (NaOH)

B.



C.

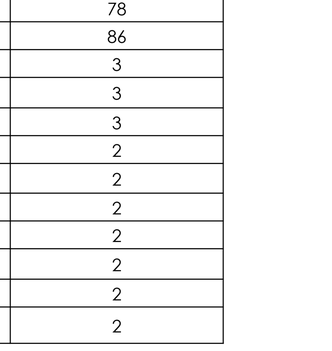


Figure 1: QPatch recordings of hKv1.3 currents
Standard solutions (**A**) and voltage protocol (**B**; applied at 0.067 Hz) were used to record the effect of venom peptides on K_V1.3 currents. The integral of current over time was measured for each voltage sweep to enable assessment of any modulatory activity in a state-independent manner (**C**). Peptides were applied as a single concentration per cell with a minimum of 5 bolus additions (2 mins per addition) to enable steady state block to be achieved.

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1. Venom library: Primary screening results

High hit rate in samples isolated from scorpion venoms

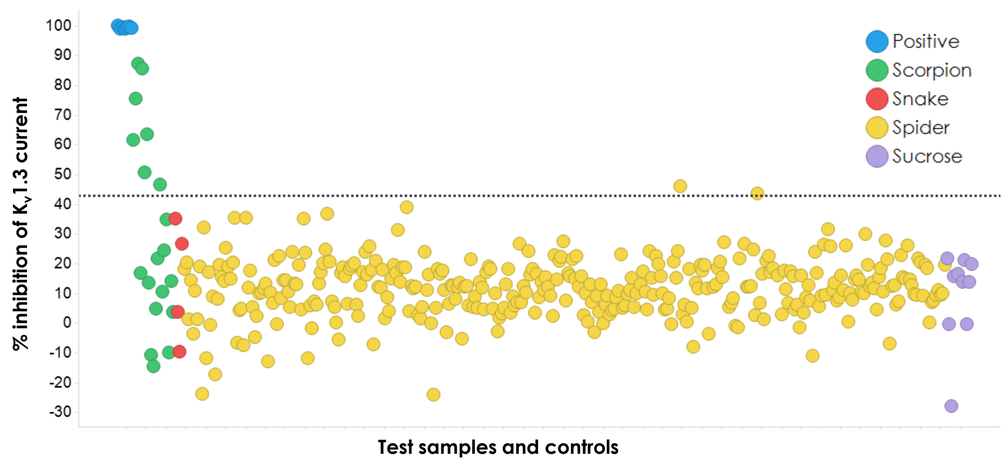


Figure 2: 370 crude venom screen
Mean % modulation data for 370 venom sample set tested at 500 ng/ml (~20 – 200 nM based on range of peptide sizes). Only extracts from scorpion species showed significant K_V1.3 inhibitory activity (cut-off >40% inhibition based on 3 x SD). Assay sensitivity defined by positive control (ShK, 50 pM) and negative control (sucrose, 5 µM).

Good hit rate from T-VDA library:
- **1.4% overall hit rate**
- **26% within scorpion species**

Sub-fractionation revealed hits within three different genus of scorpion

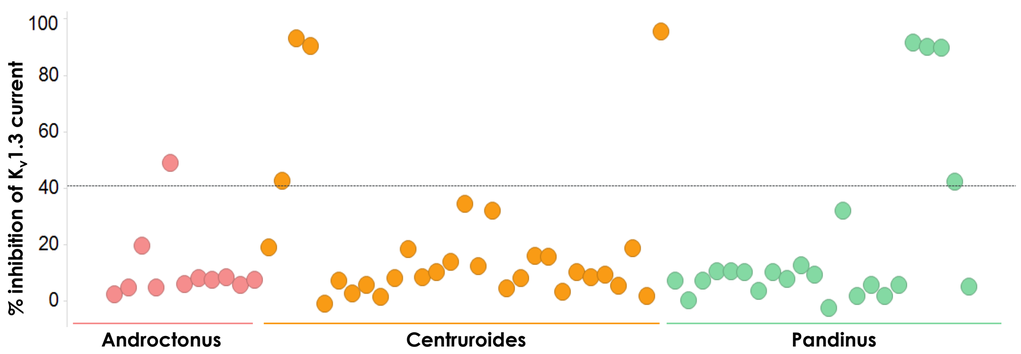
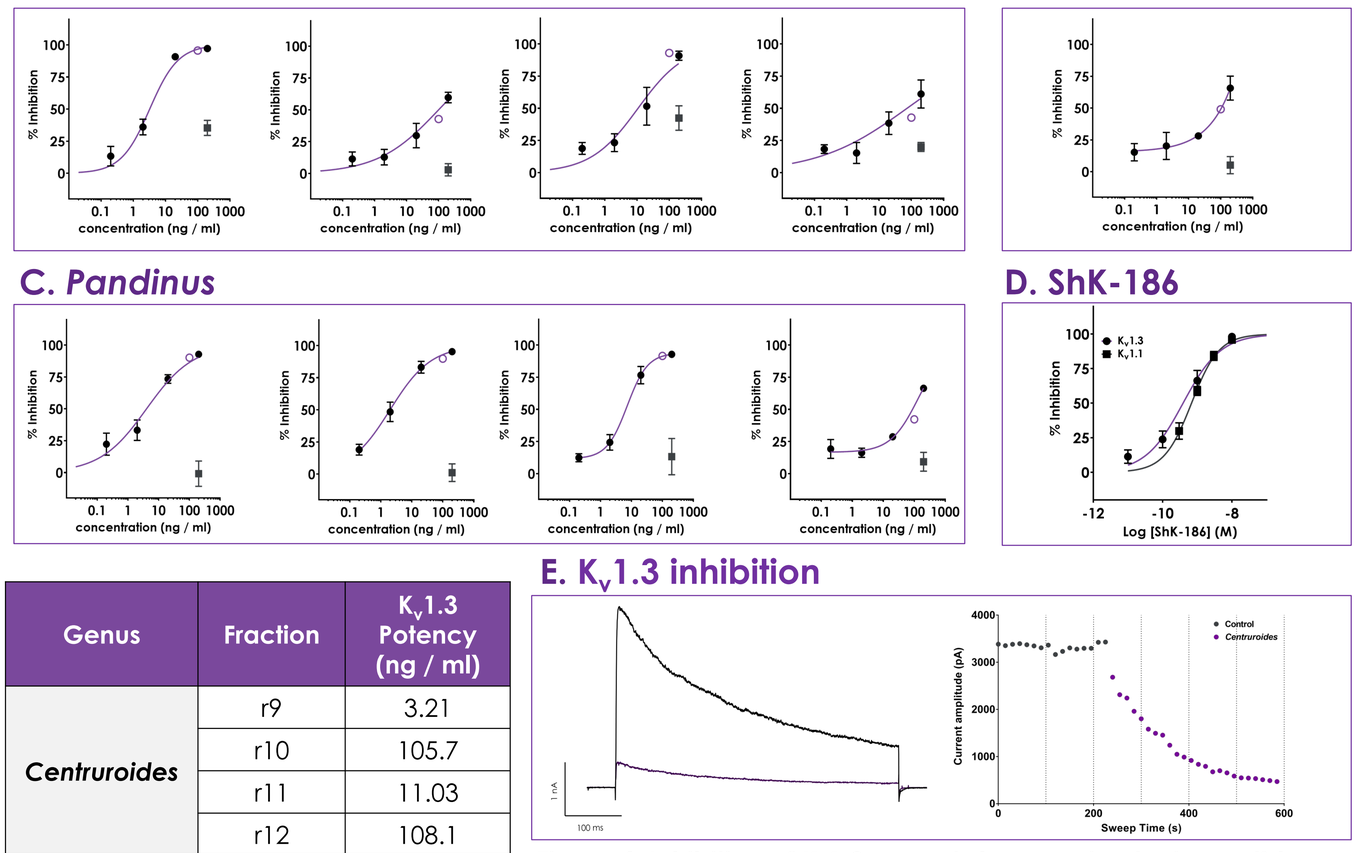


Figure 3: Active venom fractions from multiple scorpion families
Hits identified in the primary screen of samples were sub-fractionated (1 – 3 peptides) to yield 63 samples, of which 6 showed strong inhibition (>80%) of K_V1.3 current at 100 ng/ml (~10 – 100 nM based on range of peptide sizes). Overall, fractions from 4 species were active above the 40% threshold (not shown).

2. Venom fractions: K_V1.3 potency and selectivity

Hit confirmation and potency titration of novel scorpion toxin peptides



Genus	Fraction	K_V1.3 Potency (ng / ml)
Centruroides	r9	3.21
	r10	105.7
	r11	11.03
	r12	108.1
Pandinus	r5	8.87
	r6	4.02
	r7	1.90
	r8	137.7
Androctonus	r3	65.7
Stichodactyla **toxin (ShK-186)**		1.59

Figure 4: Inhibition of K_V1.3 currents by scorpion toxin peptides.
Four point concentration-response curves are shown for active venom peptide fractions from *Centruroides* (**A**), *Androctonus* (**B**) and *Pandinus* species (**C**), compared to clinical toxin candidate ShK-186 (**D**). Exemplar K_V1.3 current traces and IT on-rate kinetic plot for a *Centruroides* sample (100 ng/ml) are also shown (**E**).
Closed circles = K_V1.3 potency; Open circle = original sub/fraction %inhibition data for hit confirmation;
Grey square = inhibition of K_V1.1 currents (selectivity)

Conclusions

- Our collaboration effectively leveraged the diverse toxin fractions in Venomtech's T-VDA™ library and Metrion's automated patch clamp assays to successfully identify novel scorpion venom peptides targeting K_V1.3 channels.
- Venom library hits were reliably detected to yield novel and potent peptides.
- K_V1.x gene family selectivity of novel peptides varied between scorpion genus.
- Toxin peptides offer novel starting points for new therapeutic ligands to modulate K_V1.3 channels involved in human T-cell disease.
- Further optimisation of K_V1.3 toxin hits is possible using mutagenesis and protein engineering techniques,[3] coupling to antibody scaffolds, or use as templates for small molecule peptidomimetic approaches, to yield clinical candidates.

References

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