

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 T_{EM} cells express high levels of the voltage-gated potassium channel, $K_v1.3$, which plays a role in controlling the function of T_{EM} . 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 candidate to the clinic; partly due to a lack of potency and selectivity.

The evolutionary arms race between venomous animals and their prey has generated a diverse array of venom peptides, many of which modulate ion channels. Venom 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, which was 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 performed by Kineta.⁴ However, most $K_v1.3$ toxins, including ShK, are only moderately selective over other $K_v1.x$ and K_{Ca} gene family members that are also expressed in T-cells.

Using Venomtech's proprietary Targeted-Venom Discovery Array platform (T-VDATM) and Metrion's high quality patch clamp electrophysiology assays, we collaborated to identify novel, potent and selective venom peptide inhibitors of the human $K_v1.3$ ion 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-VDATM library were prepared by HPLC.

Group	Family	Genus	# samples
Spider	Theraphosidae	<i>Thrixopelma</i>	37
		<i>Thrixopelma</i>	36
		<i>Poecilotheria</i>	80
		<i>Pterinochilus</i>	30
		<i>Cyriopagopus</i>	78
		<i>Hysteroocrates</i>	86
Scorpion	Scorpionidae	<i>Scorpio</i>	3
	Buthidae	<i>Parabuthus</i>	3
	Buthidae	<i>Centruroides</i>	3
	Scorpionidae	<i>Pandinus</i>	2
	Theraphosidae	<i>Monocentropus</i>	2
	Buthidae	<i>Androctonus</i>	2
	Caraboctonidae	<i>Hadurus</i>	2
Hemiscorpiidae	<i>Hadogenes</i>	2	
Snake	Elapidae	<i>Naja</i>	2
		<i>Dendroaspis</i>	2

Table 1: Source of crude venom fractions used for 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 μ M) 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-186 (50 μ M) and Sucrose (5 μ M) were used as positive and negative controls, respectively.

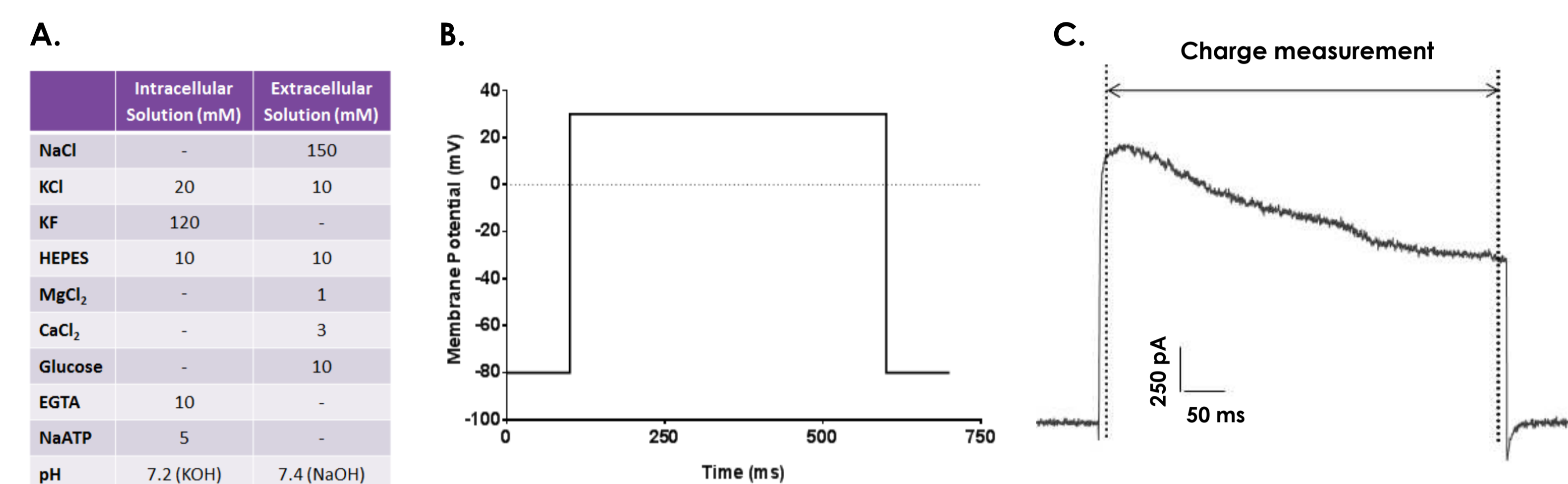


Figure 1: QPatch 48 recordings of h $K_v1.3$ current. Standard solutions (A) and voltage protocol (B; applied at 0.067 Hz) were used to record the effect of venom peptides on $K_v1.3$ current. The integral of current during the depolarising step to +30 mV was measured for each voltage sweep to enable 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 between additions) to enable steady state block to be achieved.

1. Venom library: Primary screening results

High hit rate in samples isolated from scorpion venoms

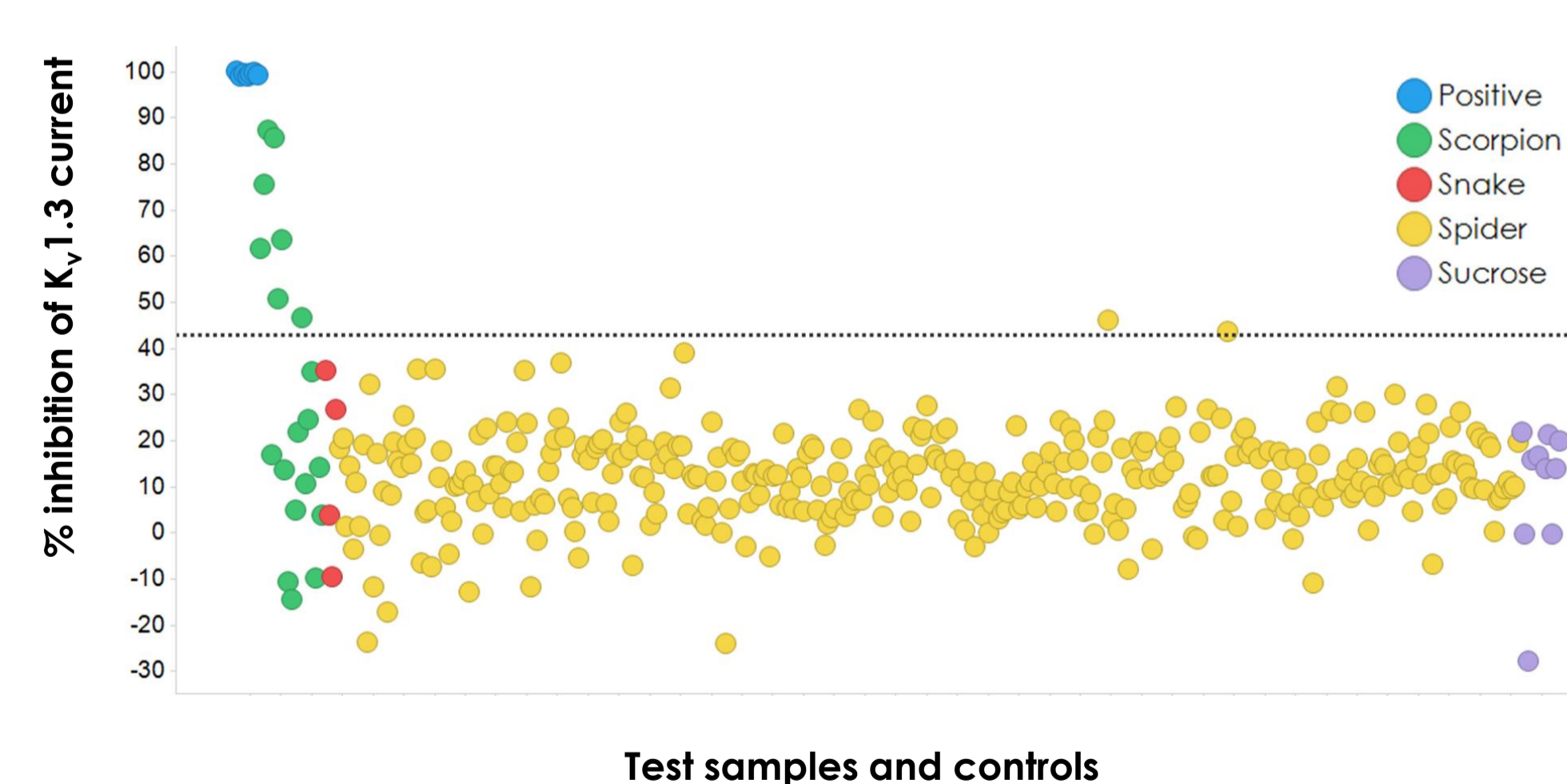


Figure 2: 370 crude venom screen. Mean % modulation data for 370 venom samples tested at 500 ng/ml (~20 – 200 nM based on a range of peptide sizes). Only extracts from scorpion species showed significant $K_v1.3$ inhibitory activity (cut-off >40% inhibition based on 3 x S.D.). Assay sensitivity defined by positive control (50 μ M ShK) and negative control (5 μ M sucrose).

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

Sub-fractionation revealed hits within three genus of scorpion

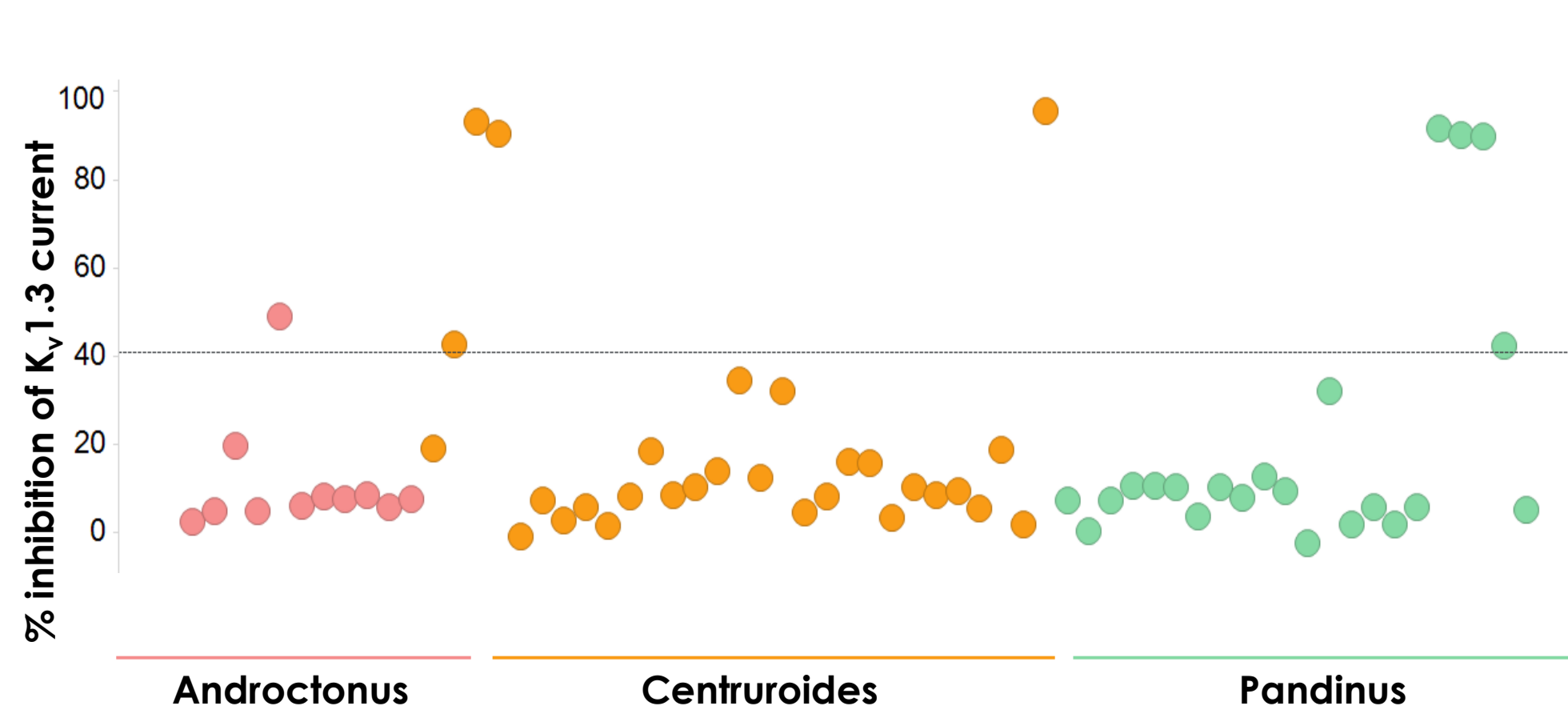
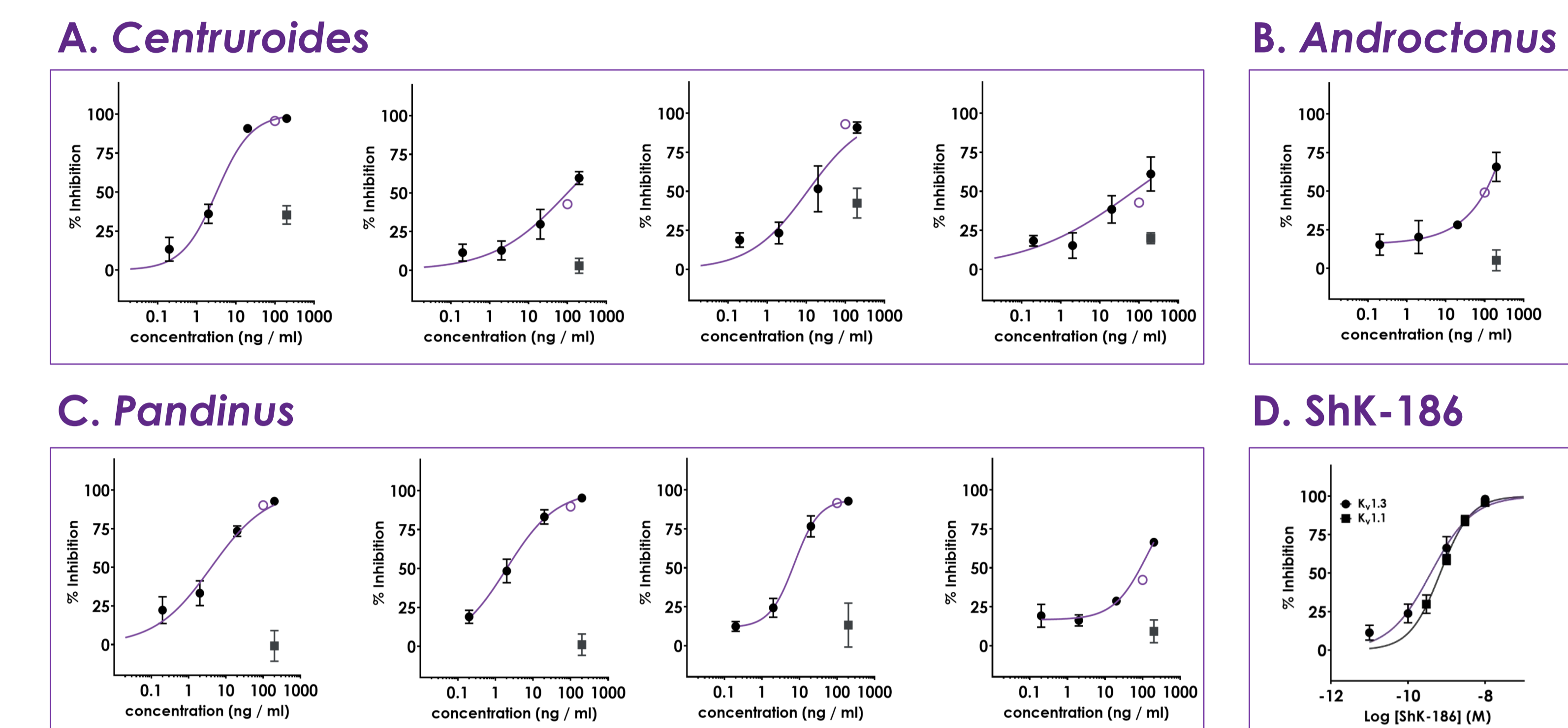


Figure 3: Active venom fractions from multiple scorpion families. Hits identified in the primary screen of samples were sub-fractionated (to 1 – 3 peptides) to yield 63 samples, of which 6 showed strong inhibition (>80%) of $K_v1.3$ current at 100 ng/ml (~10 – 100 nM based on range of peptide sizes). Overall, fractions from 4 species were active above the 40% threshold (not shown).

2. Venom fractions: $K_v1.3$ potency and selectivity

Hit confirmation & potency titration of novel scorpion toxin peptides



E. $K_v1.3$ inhibition

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
<i>Stichodactyla</i> toxin (ShK-186)		1.59

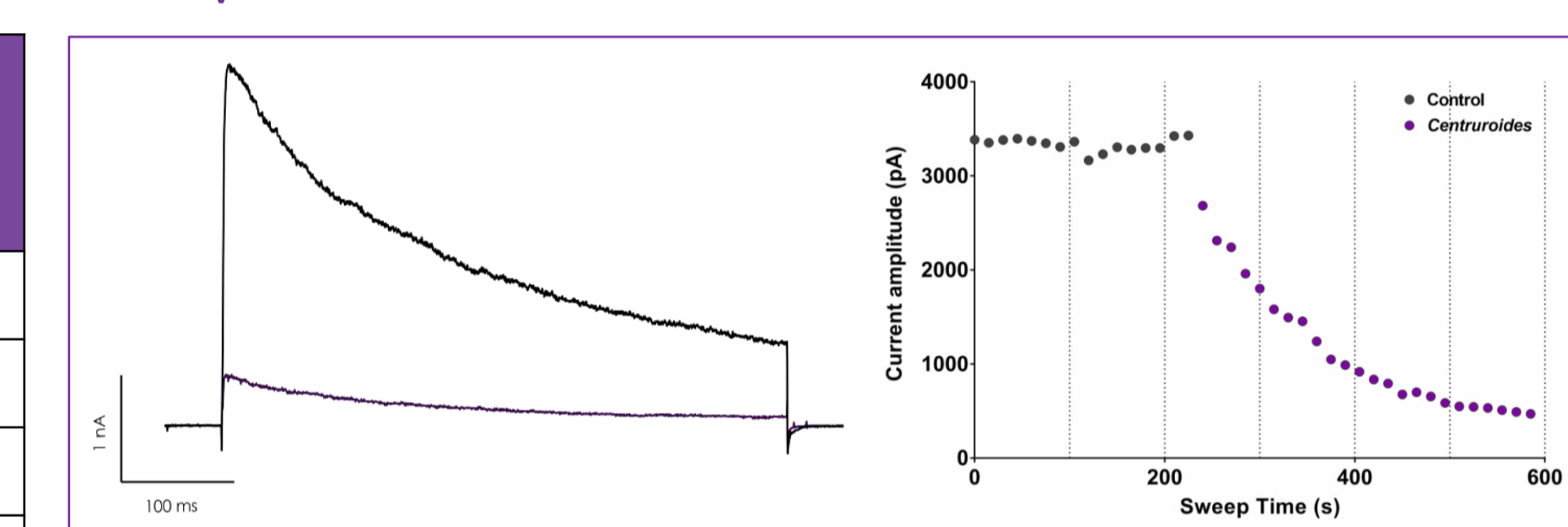


Figure 4: Inhibition of $K_v1.3$ currents by scorpion toxin peptides. Four point concentration-response curves are shown for active venom peptide fractions from *Centruroides* (A), *Androctonus* (B) and *Pandinus* species (C), compared to clinical toxin candidate ShK-186 (D). Exemplar $K_v1.3$ current 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 venom fractions in Venomtech's T-VDATM 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 inhibitors.
- $K_v1.x$ gene family selectivity of novel peptides varied between scorpion genus.
- Venom peptides offer novel starting points for new therapeutic ligands to modulate $K_v1.3$ channels involved in human T-cell disease.
- Further optimisation of $K_v1.3$ venom 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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