Development of robust, transferable, high throughput cell viability assays to discover venom peptides with selective activity against cancer cells

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Steven A Trim[1], Emily Knight[2], Alice Upton[2], Danielle McCullough[1], Charlotte Akers[2], Carol Trim[2]
[1] Venomtech Ltd. Sandwich, UK. [2] Canterbury Christ Church University, Canterbury, UK

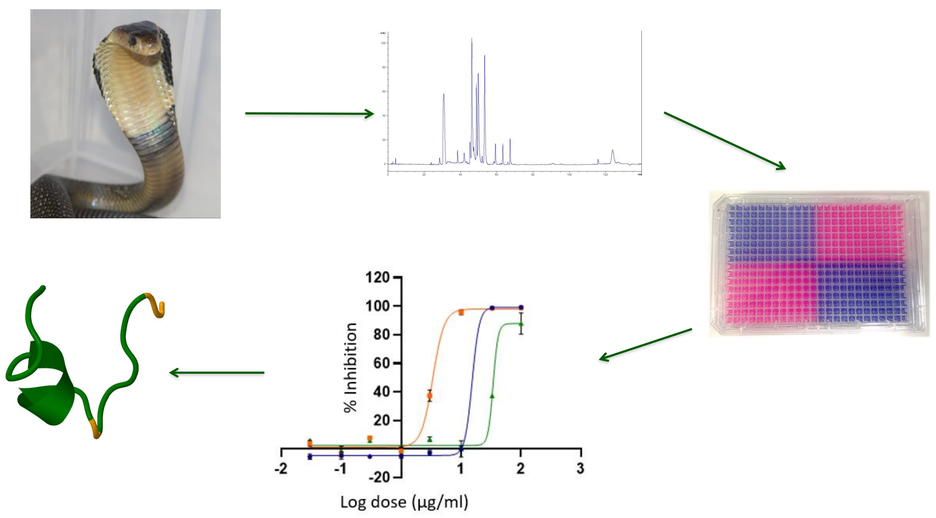
1 Overview

- To establish a simple, inexpensive and robust cell viability assay to enable medium- to high-throughput phenotypic screening of venom peptides against a variety of cancer cell types.
- This was achieved by the development of a generic resazurin assay which is simple and rapid to re-optimise for different cell lines and plate formats.
- These optimised resazurin assays were utilised to efficiently screen with fractionated venom peptides, providing hit rates of between 2-4%.
- The same assay was also used to estimate venom/ venom peptide IC_{50} values and compare potency between different cell lines to assess selectivity of venom peptides.

2 Introduction

- Cell viability assays are a key early component in phenotypic drug discovery screens where the aim is selective cytotoxicity of the disease relevant cells, whilst sparing normal cells. This phenotypic screening is target agnostic and thus facilitates discovery of new mechanisms of action and new compound classes.
- Resazurin is a redox sensitive dye that is reduced to fluorescent resorufin in the presence of viable cells of any species. Typically used in 96 well plate assays, the 384-well resazurin assay is gaining popularity, specifically with screens for antimicrobial compounds. Here we demonstrate use of this efficient, robust and cost-effective assay for anti-cancer screens against a variety of cell lines and species.
- Human cell lines (BxPC-3 pancreatic cancer, SW620 colorectal cancer and SK-OV-3 ovarian cancer) and canine cell lines (CMM26 and CMT28 mammary tumour cells and MDCK non-cancerous kidney cells) were utilised for this study.

3 Methods



- Resazurin cell viability assays optimised for human (BxPC-3, SW620 and SK-OV-3) and canine (CMM26, CMT28) tumour cell lines in 96 & 384 format.
- Venoms fractionated using UltiMate 3000 2D UHPLC (Thermo Scientific)
- Cells were dosed with 20-35 µg/ml animal venom (see Table 1 for details) and incubated for 120 minutes.
- Viability measured with 50 µl of 160 µM resazurin incubated for 120-300 minutes. Fluorescence measured (excitation 544 nm, emission 590 nm) using Fluostar Omega plate reader (BMG LABTECH).
- Dose response curves were performed on hit fractions to assess potency.

4 Results

Table 1. Summary of venom screens and dose response assays across cell lines in study

Cell Line	Format	Z'	Venom fractions screened	Screening conc. (µg/ml)	Active fractions	Hit rate (%)	Max potency (IC_{50} (µg/ml))
BxPC-3	384	0.85	303	20	11	3.63	23.64
SW620	384	0.85	303	20	9	2.97	17.85
SK-OV-3	96	0.91	92	30	3	3.26	37.23
CMM26	384	0.74	535	35	13	2.43	3.37
CMT28*	384	-	-	-	-	-	2.23

* Dose-response only

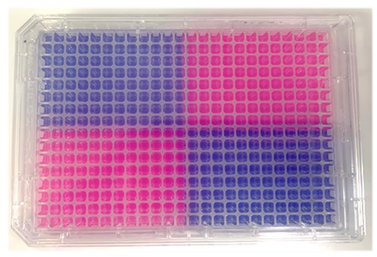


Figure 1. Example 384-well Z' plate. Pink wells indicate conversion of resazurin to resorufin by viable cells. Blue wells indicate a lack of conversion by inhibited/dead cells.

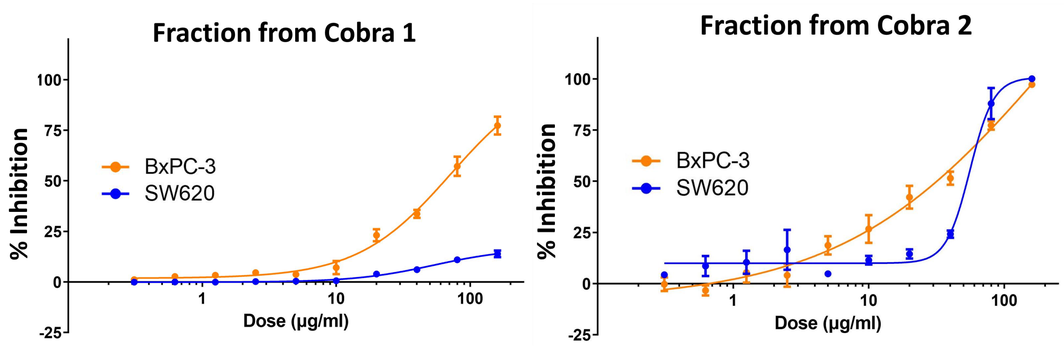


Figure 2. Dose-response curves for BxPC-3 pancreatic and SW620 colorectal human cancer cell lines

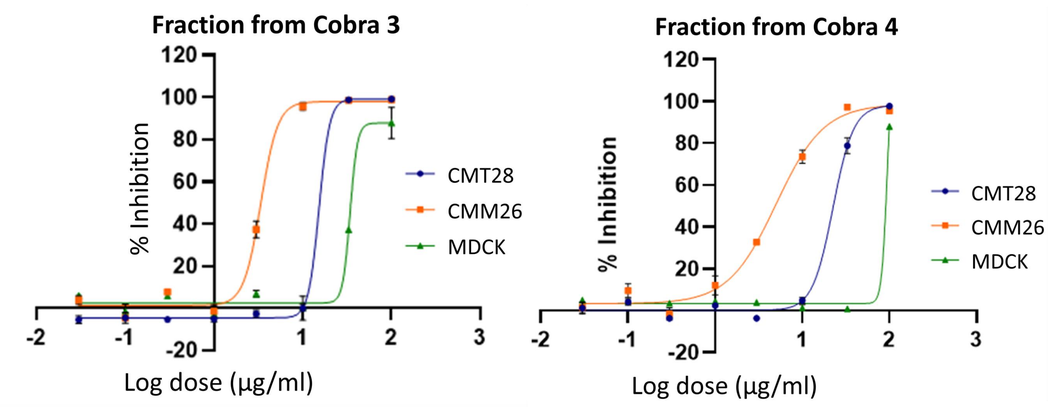


Figure 3. Dose-response curves for CMT28 and CMM26 canine mammary tumour cell lines compared with MDCK canine kidney control cell line

5 Conclusions

- The Resazurin assay detailed here is a robust and easily transferable phenotypic screening platform which may be used to rapidly and inexpensively screen in 96- and 384.
- Potent and selective venom peptides were discovered in different human and non-human cell lines.

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www.venomtech.co.uk
info@venomtech.co.uk

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