The Anti-proliferative Effects of Combretastatin Derivatives

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INTRODUCTION

- Combretastatins are a family of broad-acting chemotherapeutic drugs that inhibit cell growth by blocking the G2/M transition.
- They are already being used in a number of clinical trials, however issues remain surrounding solubility and metabolic inactivation that negatively impact the efficacy.
- Chemical modification of existing combretastatins could remedy these problems, or add additional mechanisms of action.
- Combretafuroxans are one such group of derivatives that contain a furoxan ring within their chemical structure **(Fig. 1)**. This can act as a nitric oxide donor and thus it is thought that this could act as a second hit to the cancer cells.

AIMS:

- Assess anti-proliferative activity of novel combretafuroxans RJP1 and RJP2 in breast cancer cell lines.
- Verify possible release of NO from furoxan ring as double effect

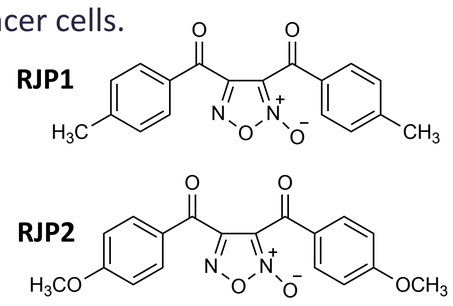


Figure 1. Chemical Structures of RJP1 and RJP2

METHODS

- Cell viability assays; cells were treated with scalar concentrations (0.006 - 100µM) of the compounds.
  - MTT assay; following 72 hour treatment, MTT was added and the resultant crystals dissolved in DMSO, before reading the absorbance on a plate reader.
  - Long treatment assay; three 72 hour treatments over the course of 10 days, crystal violet was added and the resultant crystals dissolved in methanol before reading absorbance on a plate reader.
  - Colony formation assay; low density cells treated once for 72 hours, before replacing with normal growth medium and observing growth over the next 2 weeks. As above, crystal violet was then used to measure cell viability.
- Cell cycle analysis; following 48 hour treatment the cells were trypsinised, fixed in ethanol, labelled with propidium iodide and measured on a flow cytometer.

RESULTS & DISCUSSION

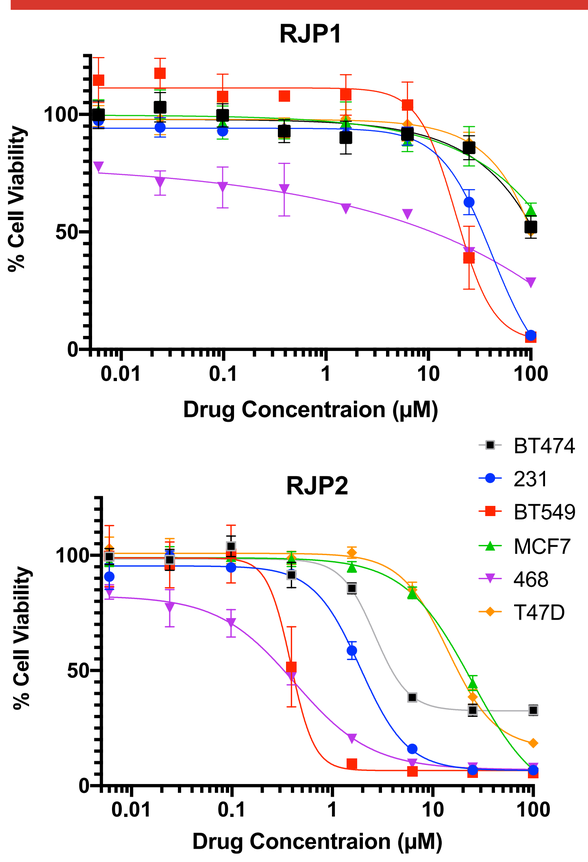


Figure 2. Effect of RJP compounds on cell viability following 72 hour treatment. IC50 values presented in table.

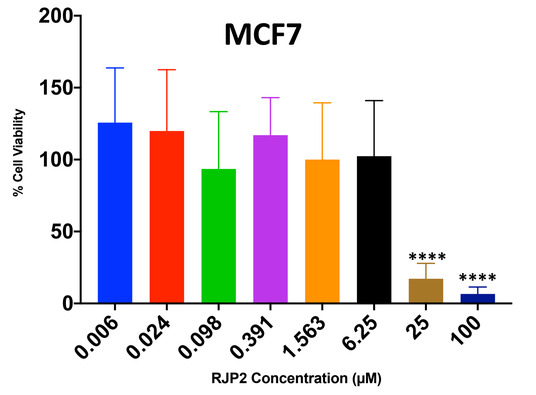
RJP treatment decreases cell viability

- The first step was to assess the cytostatic effect of the RJP compounds; do they actually decrease cell proliferation?
- RJP2 proved to be more potent than RJP1 in reducing cell proliferation and it also acted more more broadly across the cell lines tested **(Fig. 2)**.
- Interestingly the IC50s of RJP2 appeared lower in the more aggressive ER negative cell lines (231 and BT549).

Cell Line	Subtype	ER	PR	HER2	RJP1 IC50 (µM)	RJP2 IC50 (µM)
MCF7	Luminal A	+	+	-	N/A	24.46
T47D	Luminal A	+	+	-	N/A	14.19
BT474	Luminal B	+	+	+	N/A	2.645
468	Triple Negative A	-	-	-	N/A	0.4219
BT549	Triple Negative B	-	-	-	18.62	0.3865
231	Triple Negative B	-	-	-	41.85	1.904

Repeated RJP2 treatment reduces cell proliferation

- Repeated doses with RJP2 was able to limit growth at concentrations similar to the earlier IC50s **(Fig. 3)**.
- This suggests RJP2 is able to impair the activity of cancer stem cells.



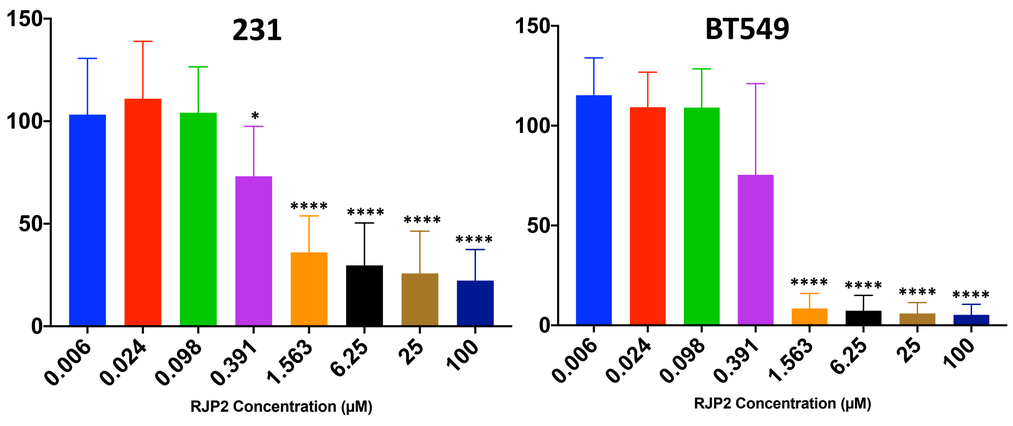
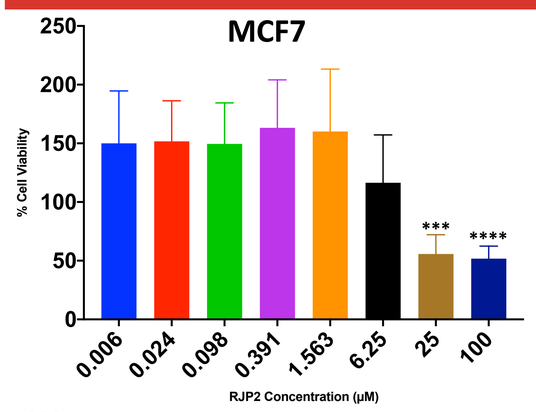


Figure 3. Effect of repeated treatments of RJP compounds on cell viability.

RESULTS & DISCUSSION (continued)



RJP2 decreases colony formation

- Higher concentrations of RJP2 reduced cell colony formation after a single dose **(Fig. 4)**.
- The ER negative cell lines were more sensitive to RJP2 treatment.
- This highlights the ability RJP2 to diminish ability of the tumour cells to sustain tumour growth.

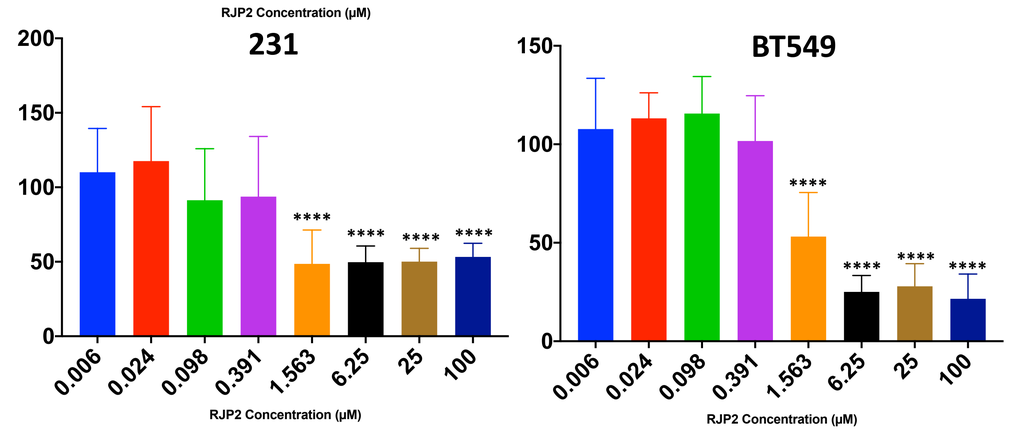


Figure 4. Effect of RJP2 on colony formation following single 72 hour treatment.

RJP compounds induce an apoptotic response

- Are the RJP compounds just restricting growth or actually killing the cells?
- Cytometry analysis further confirmed the anti-proliferative activity of both RJP compounds **(Fig. 5)**.
- Both drugs were demonstrated to induce a strong apoptotic response by increasing the subG1 fraction in two different cell lines (BT549 and 231).
- Importantly this suggests that RJP can act in a cytotoxic manner.

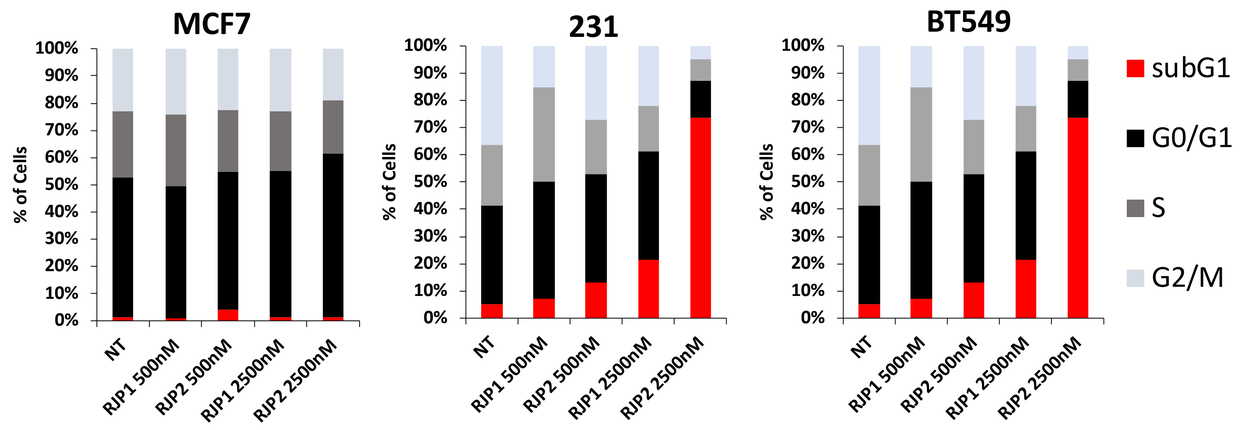
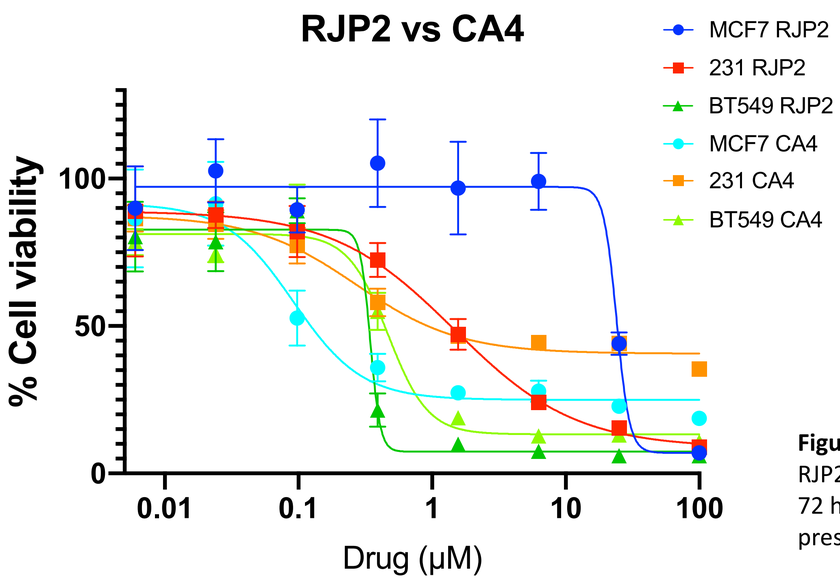


Figure 5. Effect of RJP compounds on the cell cycle following 48 hour treatment.

RJP2 compares favourably with CA4 in BT549 cells

- As the stronger candidate, how does RJP2 compare with the active Combretastatin A-4 prodrug (CA4)?
- Results from the MTT assay show that RJP2 is less potent than CA4 in MCF7 and 231 cells **(Fig. 6)**.
- However in the most aggressive cell line, BT549, it acts at a lower dose.



IC50 (µM)		
Cell Line	RJP2	CA4
MCF7	24.01	0.1021
231	1.465	0.4461
BT549	0.3445	0.4153

Figure 6. Comparison of the effects of RJP2 and CA4 on cell viability following 72 hour treatment. IC50 values presented in table.

CONCLUSION SO FAR & FUTURE PERSPECTIVES

- RJP2 appears to be a more potent agent than RJP1.
- In more aggressive (and proliferative) cell lines, the RJP compounds impair tumour growth and drive cells towards death.
- At least in BT549 cells, RJP2 does appear to be more effective than the notable CA4 derivative.

WHAT'S NEXT?

- **Cell fate analysis of cells treated with RJP compounds and CA4; timelapse microscopy can be used to determine exactly how/when cells die.**
- **Utilise a known NO detection assay to determine if extracellular NO levels do increase in the presence of RJP compounds.**

AUTHOR CONTRIBUTION

- RJP compound synthesis was performed by Dr Russell.
- Experimental plan was devised by Dr Di Leva and Dr Russell.
- Experiments were performed by Alex Cox and Dr Di Leva.