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# Antitumor activity of RP4010, a novel small-molecule inhibitor of the calcium release-activated calcium (CRAC) channel pathway

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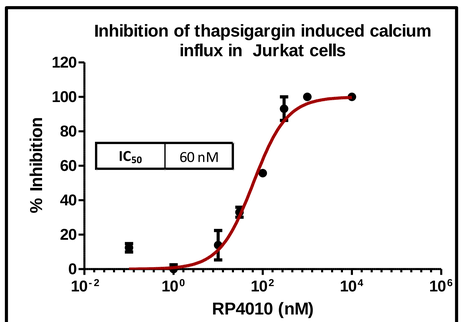
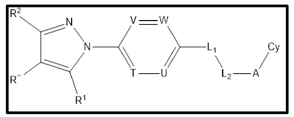
## Introduction

Store operated calcium channels namely calcium release activated calcium (CRAC) channels contribute to calcium influx in non-excitable cells. Elevation of cytosolic calcium through activation of CRAC channels mediates an array of cellular responses including metabolism and gene expression, cell growth, and proliferation. Aberrant CRAC activity has been linked to various auto-immune disorders and certain cancers *via* the NFAT pathway. Herein, we describe the preclinical profile of RP4010, a novel small molecule inhibitor of the CRAC channel pathway.

## Targeted Activity



All variables are as defined in PCT/IB2010/002539

**Figure 1.** Thapsigargin-induced calcium influx in Jurkat cells. Fluo-8 loaded cells were treated with desired concentration of inhibitors for 10 min. Release of endoplasmic reticulum calcium was induced by addition of 1 µM thapsigargin and calcium influx into cells was determined fluorimetrically.

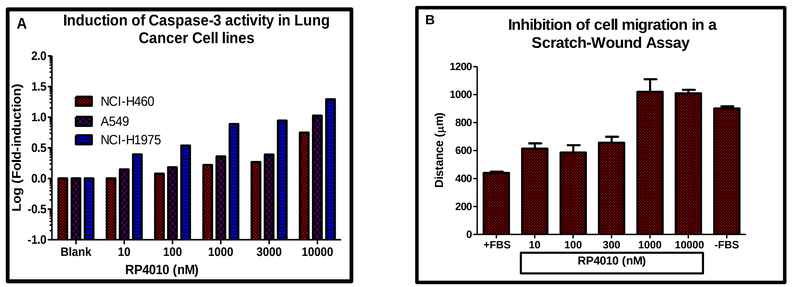






**Figure 2. A. Induction of Caspase-3 activity in NSCLC cells:** Induction of Caspase 3 by RP4010 was measured fluorimetrically. Cells were incubated with desired concentrations of the compound for 48 h. An equal number of cells per well (0.3 x $10^6$ cells) were used. Increase in apoptosis manifested by an elevation in caspase-3 levels was determined using a Caspase-3 kit from Millipore. ***A dose-dependent increase in caspase-3 was observed with RP4010***

***B. Effect of migration in A549 cells:*** A scratch was made to a serum-starved monolayer of A549 cells followed by washing and incubation with desired concentrations of RP4010 in media with 10% FBS for 72 h. The distance between the two edges of the wound was measured and % inhibition was calculated with respect to control. ***RP4010 caused a dose-dependent reduction in FBS induced migration of A549 cells thereby implicating a role for this compound in the attenuation of metastasis.***

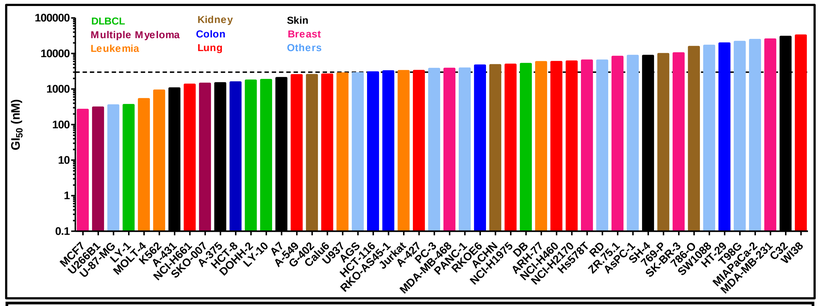



**Figure 3. Anti-proliferative effect of RP4010 across cell lines.** Cell lines were plated in media at a pre-determined cell density in 96-well plates and incubated with RP4010. After 72 h, MTT was added and $GI_{50}$ was calculated using Graphpad prism. ***Majority of the cell lines tested were sensitive to RP4010 with $GI_{50}$ ranging between 0.3 – 3 µM. Interestingly, RP4010 did not effect growth of the normal lung cell line, WI-38 indicating specificity towards cancer cells.***

| Cell Line | Apoptosis 2X Fold Induction (µM) | Apoptosis Emax | G1/S cell cycle block (µM) |
|---|---|---|---|
| PC-3 | 2.05 | 12.31 | 8.13 |
| A7 | 2.22 | 55.74 | 2.78 |
| A427 | 2.77 | 16.1 | 3.52 |
| HCT-116 | 2.97 | 20.36 | 1.89 |
| MDA MB 468 | 3.02 | 8.86 | 7.81 |
| DOHH-2 | 3.03 | 6.13 | 3.23 |
| G-402 | 3.11 | 5.14 | 3.74 |
| RKO-AS45-1 | 3.15 | 40.99 | 3.94 |
| Calu6 | 3.73 | 3.34 | 10.5 |
| DB | 4.08 | 16.8 | 7.03 |
| RKOE6 | 4.64 | 14.43 | 4.85 |
| AGS | 5.04 | 26.62 | 3.16 |
| SKO-007 | 6.42 | 15.45 | 5.2 |
| PANC-1 | 8.83 | 11.49 | 7.32 |
| 786-O | 10.31 | 4.67 | 4.4 |
| U266B1 | 11.11 | 4.88 | 3.56 |
| ACHN | 11.23 | 2.86 | 10.3 |
| RD | 11.45 | 3.03 | 7.08 |
| SK-BR-3 | 11.6 | 8.32 | 6.11 |
| SH-4 | 15.59 | 72.67 | 16.6 |
| NCI-H661 | 17.64 | 13.23 | 2.8 |
| MDA MB 231 | 18.4 | 4.35 | N/A |
| 769-P | 24.71 | 10.3 | 4.82 |
| ARH-77 | 30.74 | 5.36 | 7.02 |
| T98G | 53.96 | 21.74 | 41.3 |
| SW1088 | 75.51 | 8.54 | 33 |
| C32 | 80.52 | 4.7 | 10.7 |
| Hs 578T | N/A | 1.19 | 4.56 |
| AsPC-1 | N/A | 2.07 | 3.64 |
| Wi38 | N/A | 0.49 | 8.82 |

**Table 1. Apoptosis in cell lines treated with RP4010**. An antibody to activated caspase-3 was used to label cells from early to late stage apoptosis. The concentration of test compound that caused a 2-fold induction in the caspase-3 signal is reported, indicating a significant apoptosis induction (***Eurofins OncoPanel***).

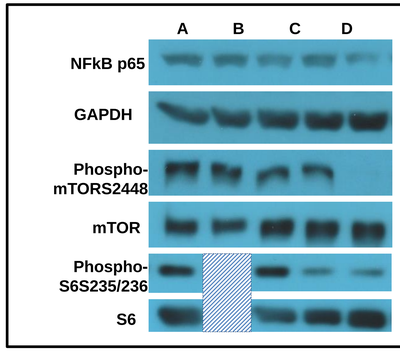



**Figure 4. RP4010 inhibits NFkB, phospho-mTOR, phospho-S6 in Jurkat**. Cell lines were plated in media at a pre-determined density in a 6-well plate in appropriate media. After overnight incubation, cells were treated with RP4010 for 24 h. Cell lysates were analyzed for the expression of the markers by Western Blotting. Gapdh, mTOR and S6 were used as a loading control for NFkB., phospho-mTOR, and phospho-S6 respectively. **Lanes**: **A**: Blank ; **B**: 10 nM; **C**: 100 nM; **D**: 1000 nM; **E**: 3000 nM; ***Treatment with RP4010 caused a dose-dependent reduction in NFkB, phospho-mTOR, phospho-S6 in Jurkat cells.***

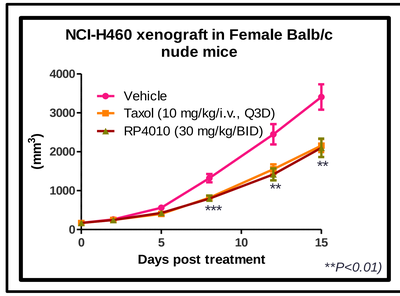



**Figure 5. Anti-tumor effect of RP4010 in Female Balb/c Nude Mice Bearing NCI-H460 Human Non-Small Cell Lung Cancer Xenografts.** RP4010 (30 mg/kg/BID) did not effect body weights.Treatment with RP4010 resulted in significant inhibition of tumor growth in animals bearing NCI-H460 human non-small cell lung tumor xenografts. The tumor growth inhibition (TGI) value on Day 15 was 36.7%, and 38.3%, for Taxol and RP4010, respectively.

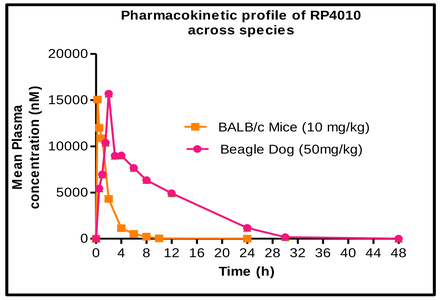



**Figure 6. Single dose pharmacokinetic profile of RP4010 in mice, rat, and dog.** Compound was administered orally as a suspension followed by blood collection across a 24-h period. Plasma was harvested and analyzed for RP4010 concentrations by LC-MS/MS. RP4010 was rapidly absorbed reaching up to 15 µM concentrations at the doses tested

## SUMMARY & CONCLUSIONS

- RP4010 is a potent inhibitor of CRAC channel function with subsequent inhibition of downstream NFAT activity
- Demonstrated activity in several cell lines representative of solid tumors and hematological malignances
- Marked inhibition of oncogenic markers downstream with favourable pharmacokinetics *in vivo*
- A Phase-1 trial (NCT03119467) in patients with relapsed or refractory Non-Hodgkin Lymphoma is currently ongoing in USA

*AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics (2017)*

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