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Targeting lipid kinase PIP5K1α as a promising strategy for the treatment of castration-resistant prostate cancer

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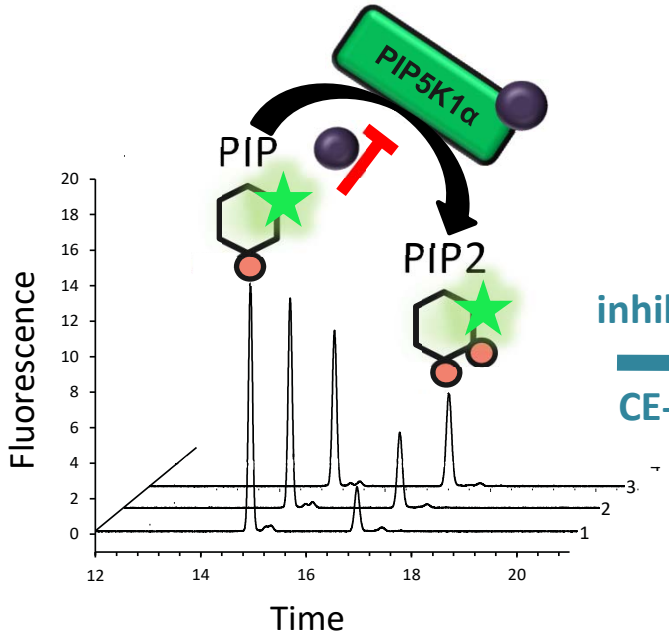
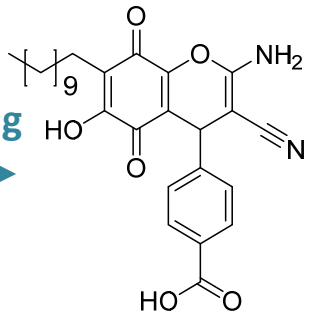
Targeting lipid kinase PIP5K1α as a promising strategy for the treatment of castration-resistant prostate cancer

Graphical Abstract



inhibitor screening

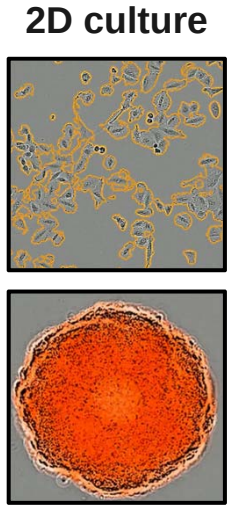
CE-based assay



IC_{50} = 1.55 μM

anticancer effects

live cell imaging



Abstract:

Phosphoinositides are a family of tiny cellular lipids formed through a series of phosphorylation processes catalyzed by enzymes termed phosphatidylinositol-phosphate kinases (PIPKs). The phosphatidylinositol 4-phosphate 5-kinase type 1 α (PIP5K1α) is the main isoform responsible for generating membrane pools of phosphatidylinositol-4,5-bisphosphate (PIP2), which, in turn, serves as a substrate for the well-established cancer-relevant target, phosphatidylinositol 3-kinases (PI3Ks). Recent studies provide strong evidence for a key role of PIP5K1α isoform in the development of prostate and breast cancers indicating that targeting this kinase could offer an effective therapeutic strategy in certain types of cancer.

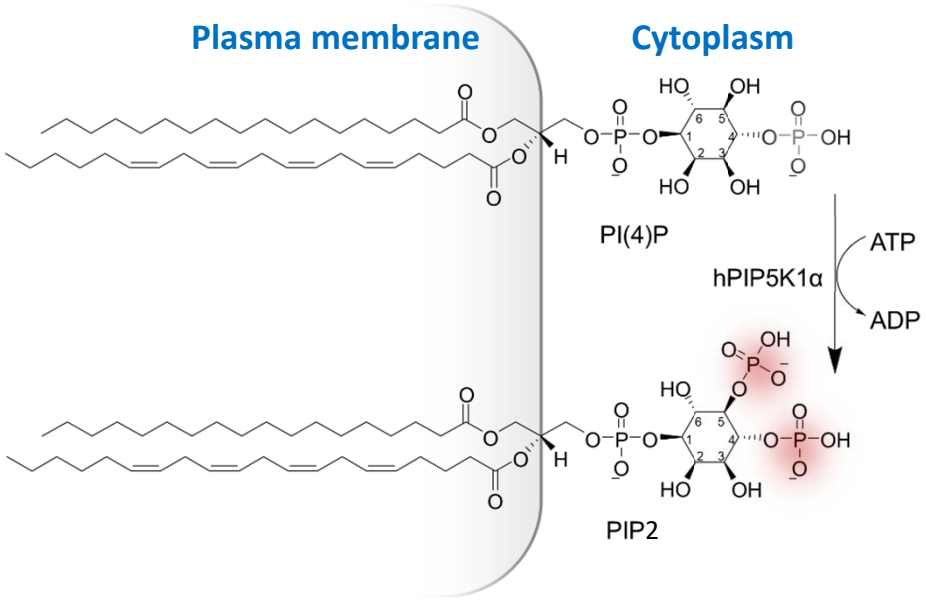
To identify small molecules that can directly inhibit the catalytic activity of PIP5K1α, we developed a robust nonradiometric assay to determine the activity of recombinantly expressed human PIP5K1α in bacterial cell lysates. This assay is based on the separation of a fluorescently labelled PIP5K1α substrate and its corresponding enzymatically phosphorylated product by capillary electrophoresis (CE).

Here, a compound with a 2-amino-3-cyano-4H-pyranobenzoquinone scaffold is presented as an example of potent inhibitors of human PIP5K1α identified recently in our lab using the developed CE-based assay. This compound exhibited potent inhibitory effect on PIP5K1α activity with an IC_{50} value of 1.55 µM, in a substrate-competitive mode of action. Furthermore, its ability to induce anticancer effects in 2D and 3D cell culture experiments was evaluated. The identified compound may provide the basis for developing highly potent and selective inhibitors of PIP5K1α in cancer cells.

Keywords: capillary electrophoresis; enzyme inhibitor; lipid kinase; PIP5K1α; prostate cancer

Introduction-Phosphoinositides

- Essential polyphosphorylated lipids docked in eukaryotic cell membrane
- Biogenesis mediated by a family of lipid kinases, phosphatidylinositol-phosphate kinases (PIPKs)
- PIP5K1α responsible for generating membrane pools of PIP2 from PI(4)P
- PIP2 is involved in several cell signalling pathways

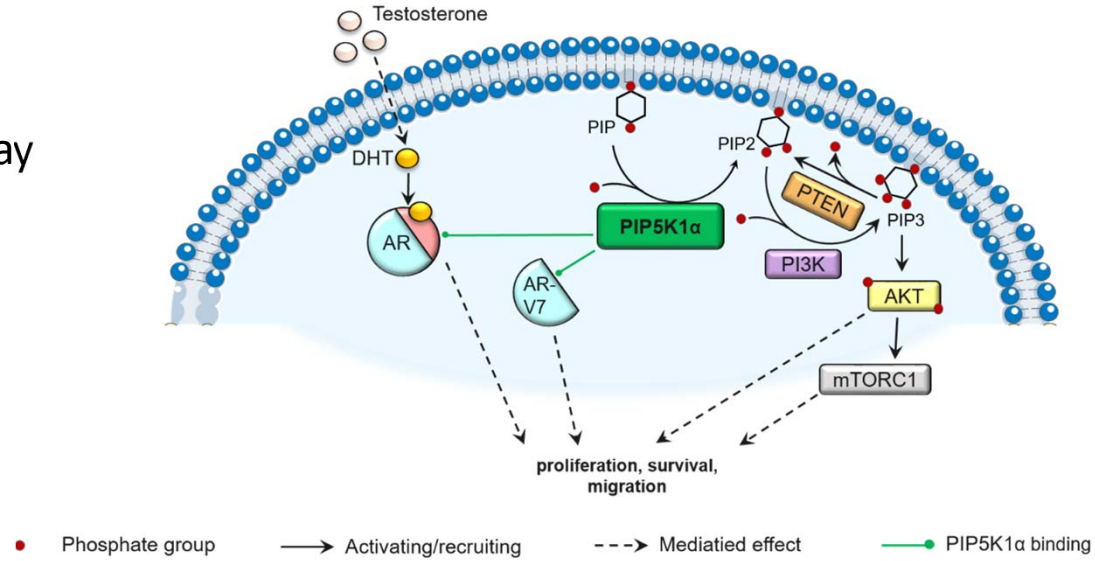


PI(4)P: phosphatidylinositol-4-phosphate
PIP2: phosphatidylinositol-4,5-bisphosphate
hPIP5K1: human phosphatidylinositol 4-phosphate 5-kinase type 1 α (PIP5K1α)

Introduction- PIP5K1α as a target in treatment of castration-resistant prostate cancer (CRPC)

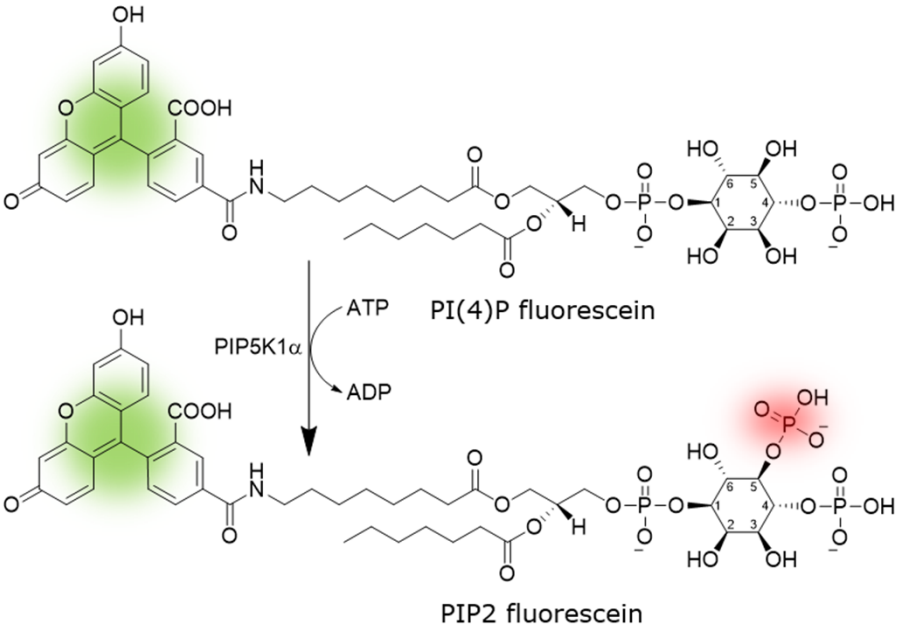
- Upstream activator of PI3K through modulating PI3K/AKT/mTOR pathway
- Potential crosstalk with androgen receptor (AR)-mediated signalling
- overexpression is reported in aggressive forms of prostate and breast cancers

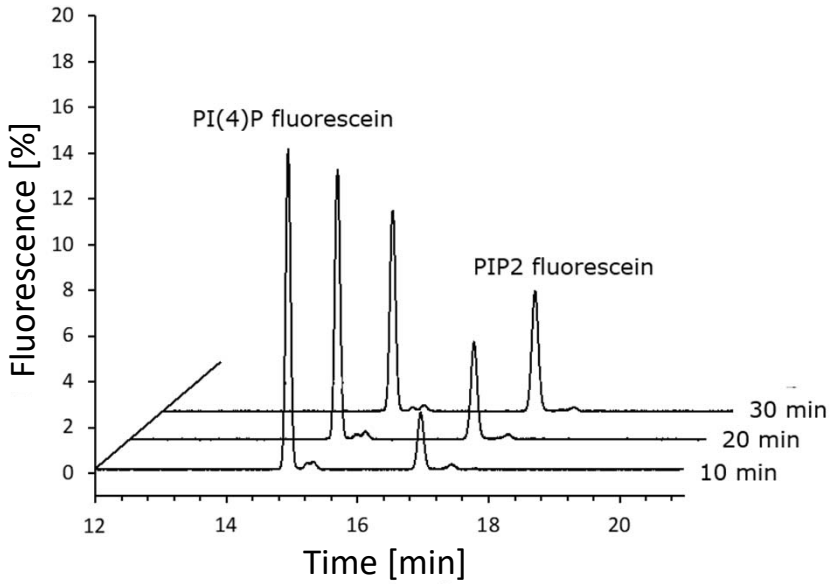
➡ **PIP5K1α plays important roles in growth and invasion of malignant prostate (and possibly other) tumors**



PTEN: phosphatase and tensin homologue; **PI3K:** phosphatidylinositol 3-kinase; **AKT:** protein kinase B; **mTORC1:** mammalian target of rapamycin complex 1; **DHT:** dihydrotestosterone; **AR:** androgen receptor; **AR-V7:** constitutively active AR variant V7

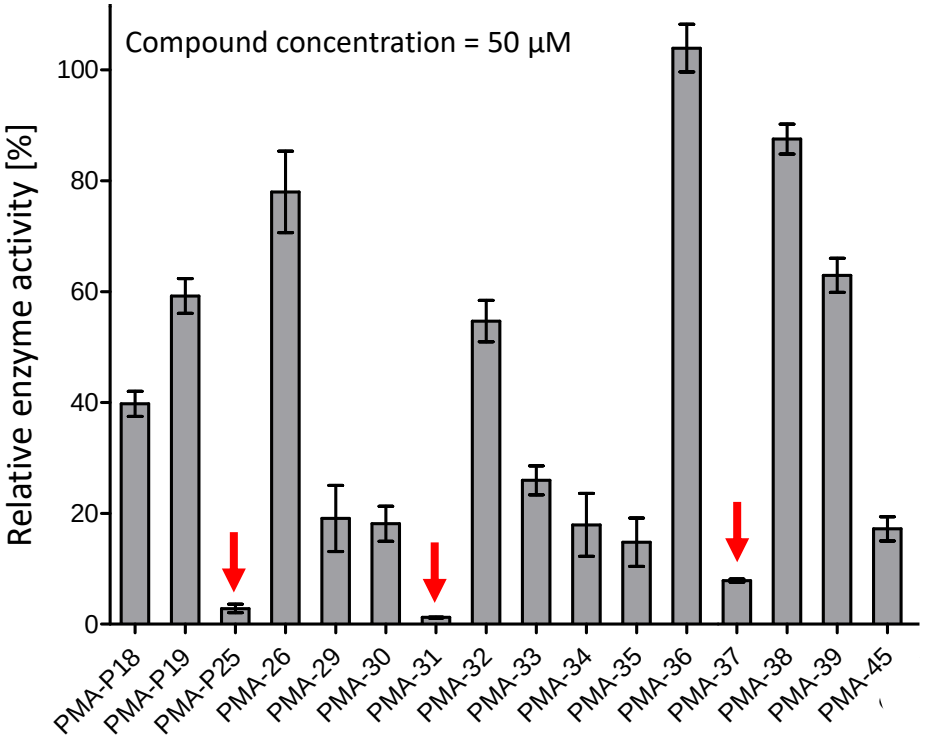
Results and discussion-Capillary electrophoresis-based kinase activity assay for PIP5K1α

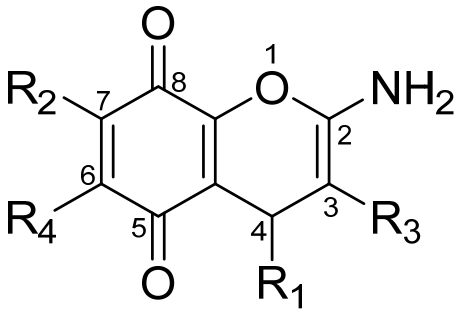




For detailed inforamtion on the detailed assay setup please refer to
Strätker, K. *et al. FEBS J*, **287**: 3042-3064 (2020)

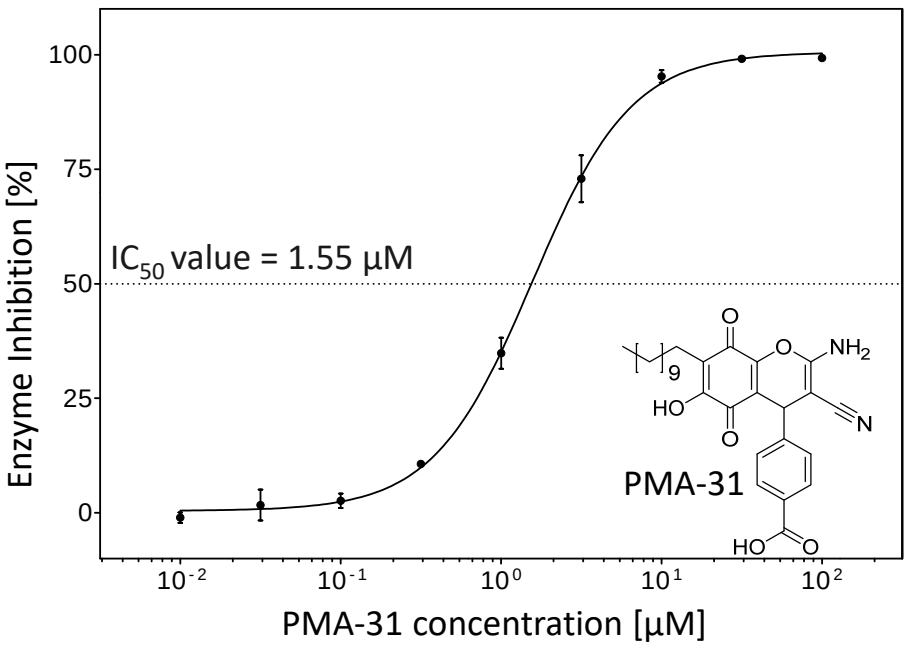
Results and discussion-Screening of Pyranobenzoquinone derivatives for inhibitors of human PIP5K1α

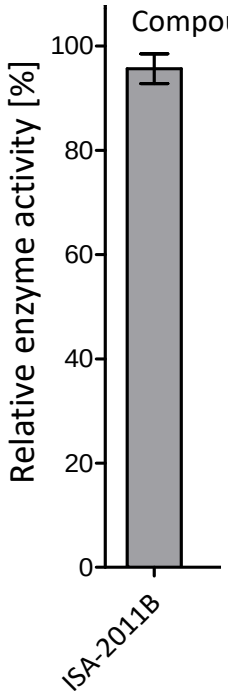




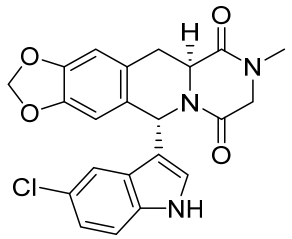
For detailed inforamtion on the structural features of the compounds tested please refer to Strätker, K. *et al. FEBS J*, **287**: 3042-3064 (2020)

Results and discussion-IC_{50} determination of PMA-31





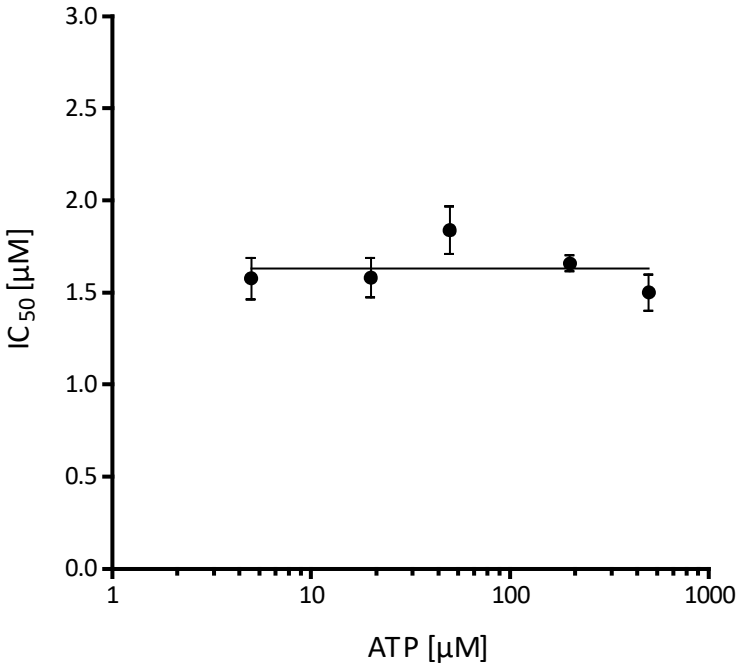
diketopiperazine fused C-1 indol-3-yl substituted 1,2,3,4-tetrahydroisoquinoline

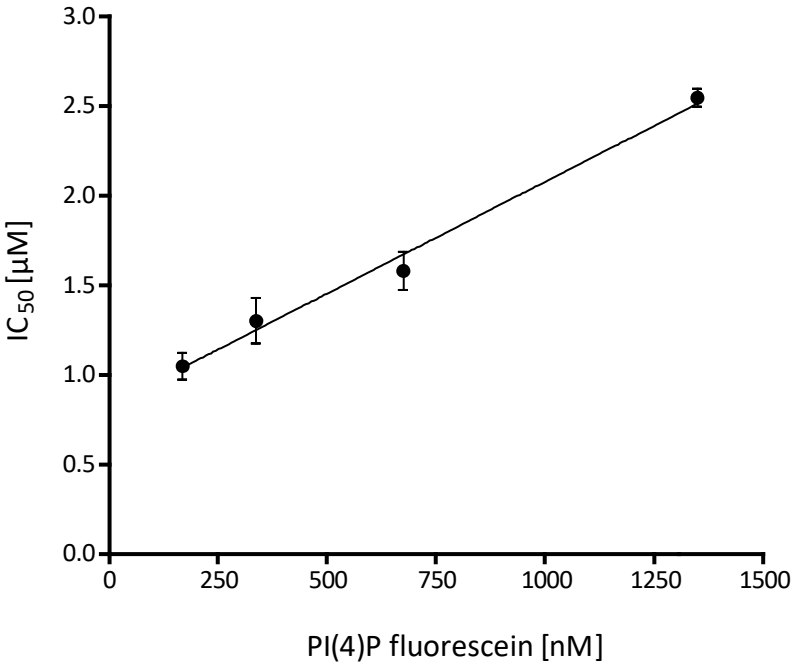


ISA-2011B

- **PMA-31 is the most potent direct inhibitor of the catalytic activity of PIP5K1α identified in the screen**
- **The recently reported PIP5K1α inhibitor (ISA-2011B) does not directly inhibit the catalytic activity of the enzyme**

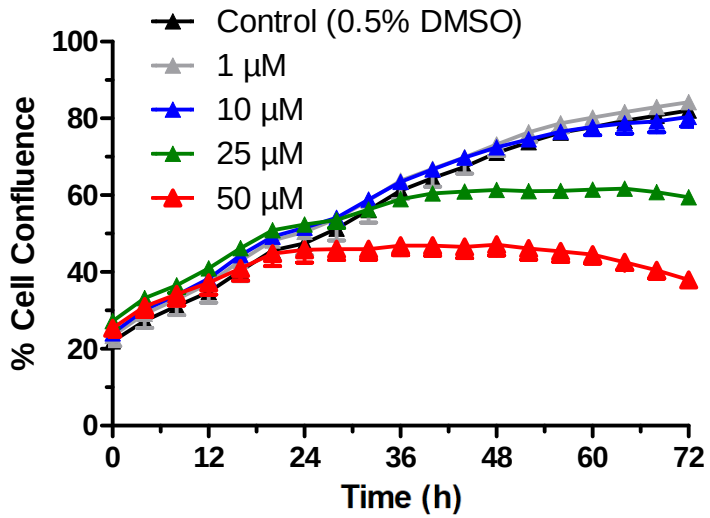
Results and discussion-Investigation of the mode of inhibition of PMA-31

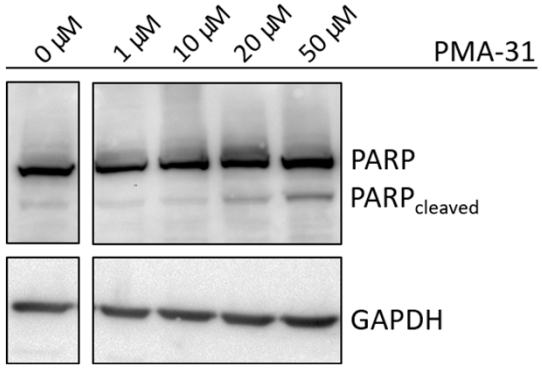


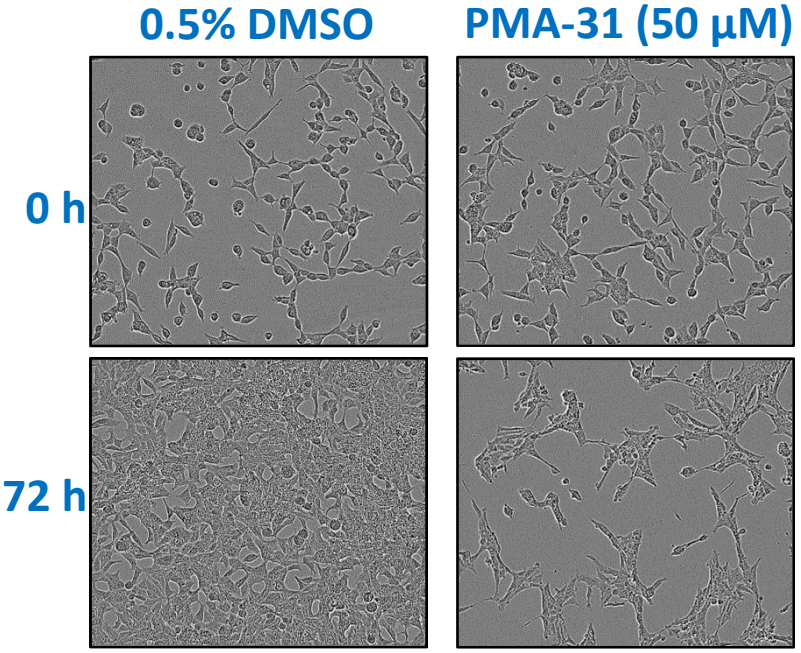


PMA-31 does not inhibit ATP binding to PIP5K1α but rather exhibits a substrate-competitive inhibition of PIP5K1α

Results and discussion-Effects of PMA-31 on LNCaP prostate cancer cell line

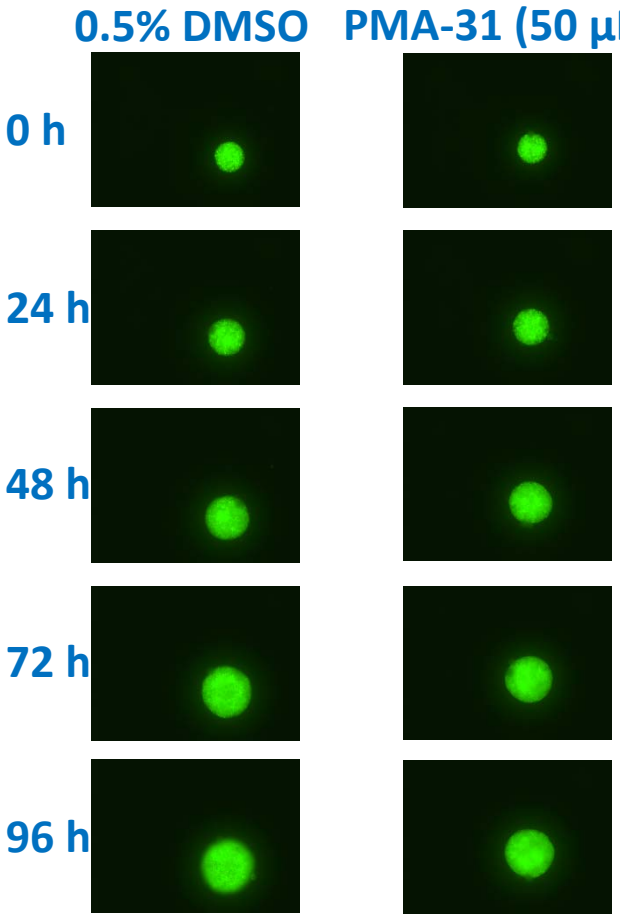


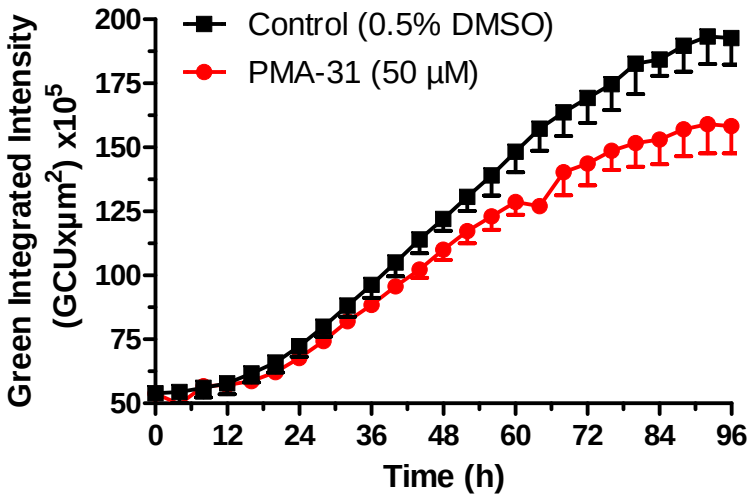




PMA-31 inhibits the proliferation and induces apoptosis of 2D culture of LNCaP cells in a dose-dependent manner

Results and discussion-Effect of PMA-31 on the growth of spheroids of breast cancer cells





PMA-31 inhibits the growth of MCF7 green spheroids with a delayed onset of inhibition

Conclusions

- Small molecule inhibitors of PIP5K1α can be identified using the developed capillary electrophoresis-based kinase assay
- The pyranobenzoquinone derivative PMA-31 represents the first direct inhibitor of PIP5K1α catalytic activity with IC_{50} = 1.55 µM
- PMA-31 does not compete with ATP for its binding site but acts as a substrate competitive inhibitor
- PMA-31 exhibits moderate effects on prostate and breast cancer cell lines in 2D and 3D cell culture formats,respectively
- Optimization of the identified compound may lead to developing new class of highly potent and selective inhibitors of PIP5K1α in cancer cells