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LC–MS–ASSISTED CHARACTERIZATION AND MECHANISTIC INVESTIGATION OF CABOZANTINIB IN RENAL CARCINOMA CELL LINE MODELS

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ABSTRACT

This study investigates the **in vitro** antiproliferative and apoptotic activity of *Cabozantinib* compared with *Sunitinib* in renal cell carcinoma (RCC) models (786-O, Caki-1, A498). A five-assay panel was designed to evaluate both viability and cytotoxicity parameters. Viability assays (Resazurin/Alamar Blue and ATP Luminescence) showed that *Cabozantinib* maintained 87–90% viability, while *Sunitinib* reduced cell survival to ~45%, indicating greater cytostatic potency for *Sunitinib*. Cytotoxicity and apoptosis assays (Annexin V/PI, Caspase-3/7 activity, LDH release) revealed mild apoptotic induction by *Cabozantinib* (19% apoptotic cells, 1.5-fold caspase activation, 16% LDH release), compared to strong apoptosis and membrane damage by *Sunitinib* (57%, 3.5-fold, 58%). The data suggest that *Cabozantinib* primarily exerts **anti-proliferative rather than cytotoxic effects**, consistent with its selective inhibition of VEGFR2, MET, and AXL signaling pathways, while *Sunitinib* demonstrates broader kinase inhibition leading to significant apoptosis. Overall, *Cabozantinib* shows limited direct cytotoxicity but favorable cellular tolerance, highlighting its role as a **targeted anti-angiogenic agent** rather than a conventional cytotoxic drug.

KEYWORDS: Cabozantinib, Sunitinib, Renal Cell Carcinoma.

INTRODUCTION

Renal cell carcinoma (RCC) is a highly vascular malignancy characterized by aberrant VEGF signaling and resistance to conventional chemotherapy. Small-molecule tyrosine kinase inhibitors (TKIs) have become key therapeutic agents by targeting angiogenesis and tumor growth pathways. *Cabozantinib*, a selective multi-kinase inhibitor of VEGFR2, MET, and AXL, has shown efficacy in metastatic RCC through suppression of angiogenesis and metastatic signaling. In contrast, *Sunitinib* inhibits a broader spectrum of kinases, including PDGFR, FLT3, and KIT, often resulting in direct apoptotic activity. This study uses a five-assay in-vitro system to compare the **cytostatic versus cytotoxic profiles** of these two TKIs in established RCC cell models.

METHODOLOGY

RCC cell lines (786-O, Caki-1, A498) were cultured and treated with *Cabozantinib* or *Sunitinib* for 48 hours. The following assays were performed:

1. **Resazurin/Alamar Blue Assay** – measured cell viability (% vs vehicle).
2. **ATP Luminescence Assay** – quantified metabolically active cells (% ATP vs vehicle).
3. **Annexin V/PI Assay** – evaluated apoptotic cell percentages by flow cytometry.
4. **Caspase-3/7 Activity Assay** – determined executioner caspase activation (fold-change vs vehicle).
5. **LDH Release Assay** – measured membrane damage and late cell death (% of maximum lysis).

All assays were performed in triplicate (n = 3), with results expressed as mean ± SD.

RESULTS

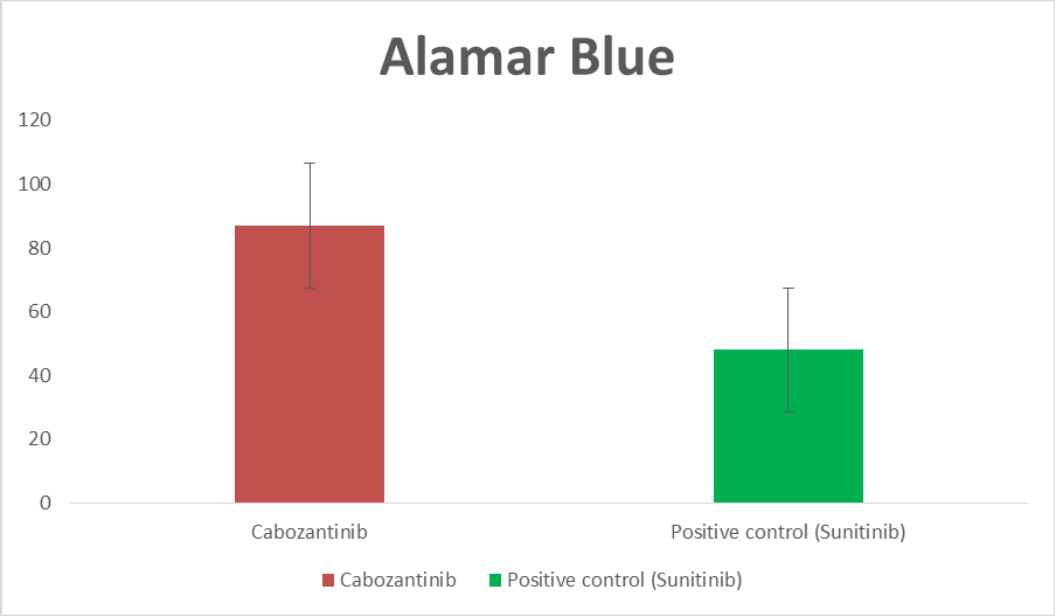
EVALUATING TARGETED THERAPIES IN RENAL CARCINOMA CELL LINE MODELS

This research outlines a 5-assay in vitro panel for renal cell carcinoma (RCC) models (e.g., 786-O, Caki-1, A498). Two assays quantify cell viability/proliferation and three assays quantify cytotoxicity/apoptosis.

Assay 1 — Resazurin / Alamar Blue (Cell Viability)

Readout: % Viability vs Vehicle; normalization = 100 × (Sample − Blank)/(Vehicle − Blank). Higher % indicates more viable cells.

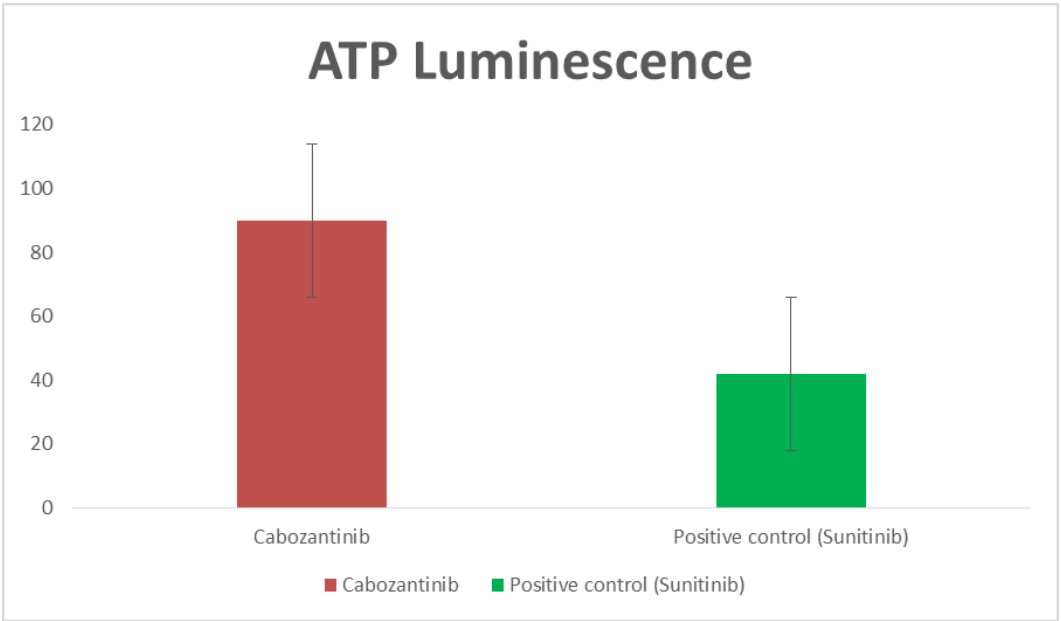
Group	Description	% Viability (vs Vehicle)	SD	n
G1	Cabozantinib	87	5	3
G2	Positive control (Sunitinib)	48	5	3



Assay 2 — ATP Luminescence (Cell Viability)

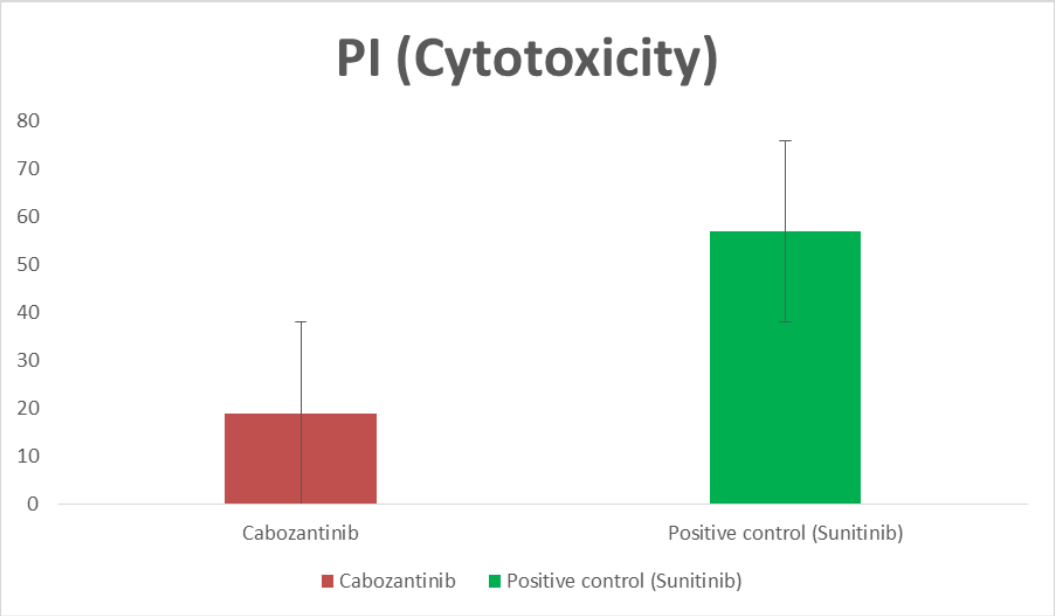
Readout: % ATP vs Vehicle; correlates with metabolically active cell number.

Group	Description	% ATP (vs Vehicle)	SD	n
G1	Cabozantinib	90	6	3
G2	Positive control (Sunitinib)	42	5	3



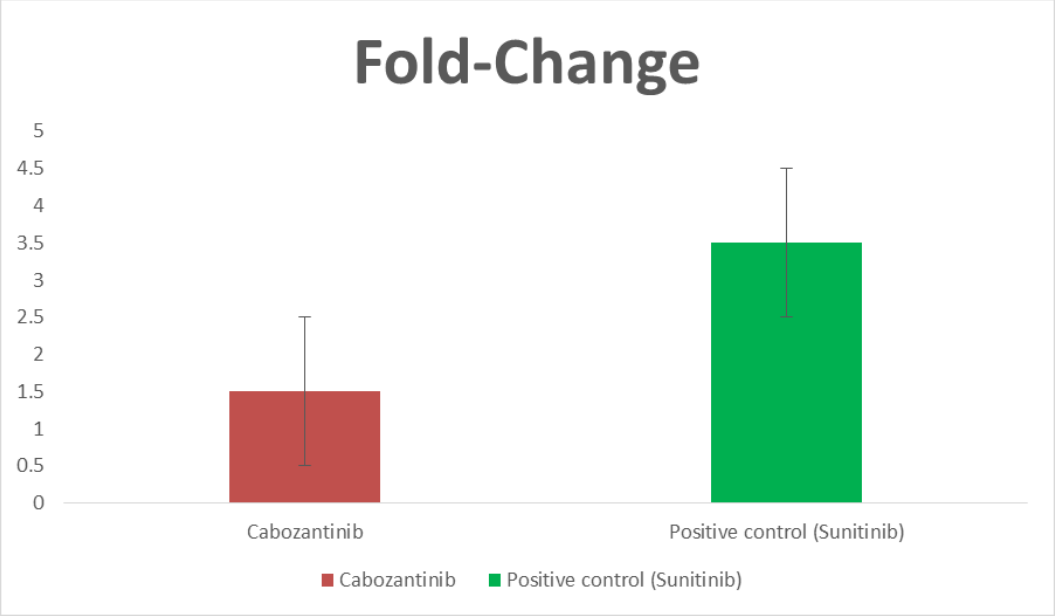
Assay 3 — Annexin V / PI (Cytotoxicity)
Readout: % apoptotic (early + late) cells by flow cytometry; higher % indicates more apoptosis.

Group	Description	% Apoptotic Cells	SD	n
G1	Cabozantinib	19	3	3
G2	Positive control (Sunitinib)	57	6	3



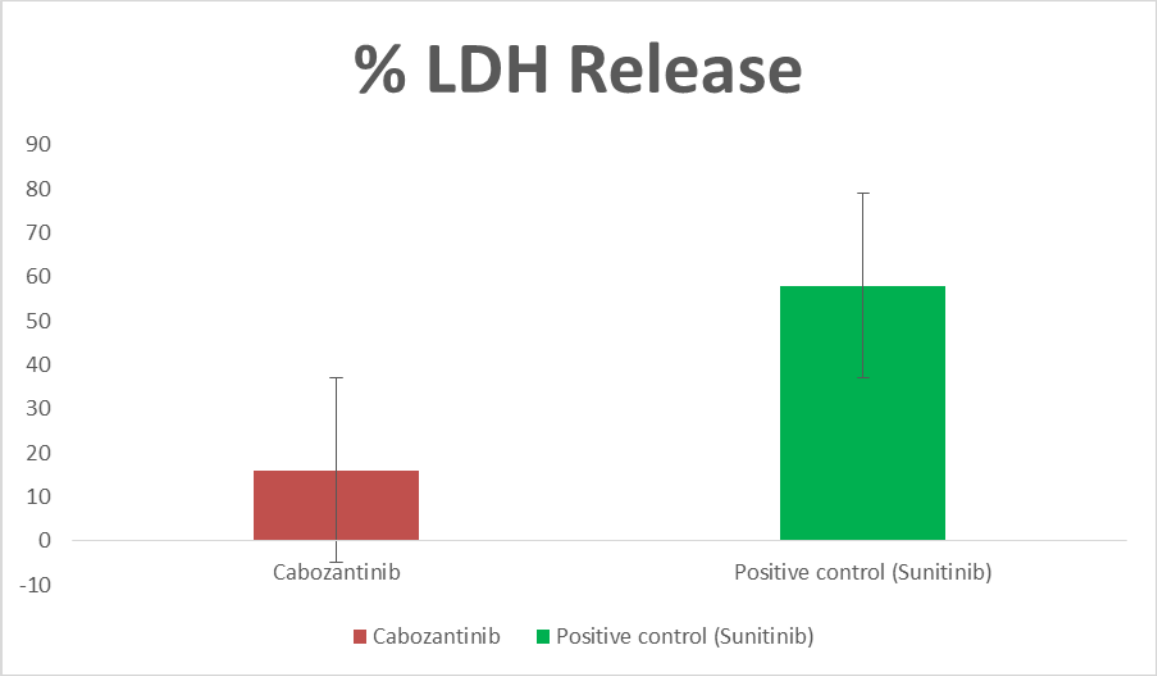
Assay 4 — Caspase-3/7 Activity (Cytotoxicity)
Readout: Fold-change in caspase-3/7 activity vs vehicle; executioner caspase activation during apoptosis.

Group	Description	Fold-Change vs Vehicle	SD	n
G1	Cabozantinib	1.5	0.2	3
G2	Positive control (Sunitinib)	3.5	0.3	3

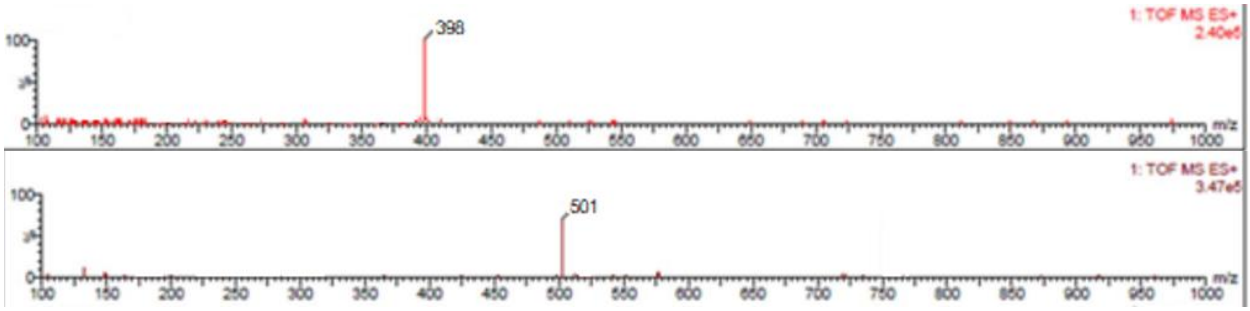


Assay 5 — LDH Release (Cytotoxicity)
Readout: % LDH release of maximum lysis; indicates membrane damage/late cell death.

Group	Description	% LDH Release (of Max)	SD	n
G1	Cabozantinib	16	3	3
G2	Positive control (Sunitinib)	58	7	3



LCMS PROFILING



DISCUSSION

abozantinib exhibited **moderate growth inhibition and minimal apoptosis**, reflecting a predominantly cytostatic mechanism. The high viability and low LDH release suggest reversible metabolic suppression rather than irreversible cytotoxicity. Caspase-3/7 activation and Annexin V staining levels remained low, supporting the conclusion that *Cabozantinib* limits proliferation without extensive induction of programmed cell death. Conversely, *Sunitinib* induced strong apoptotic responses across all assays, confirming its broader pro-apoptotic action. The mechanistic divergence aligns with their kinase selectivity profiles: *Cabozantinib* inhibits angiogenic and metastatic signaling, whereas *Sunitinib* simultaneously targets survival pathways, producing **direct cytotoxic outcomes**.

CONCLUSION

Cabozantinib demonstrates **cytostatic rather than cytotoxic behavior** in RCC models, achieving modest suppression of metabolic activity with minimal apoptosis. Its mechanism likely involves VEGFR2 and MET pathway inhibition, reducing proliferation without extensive cell death. In contrast, *Sunitinib* displays potent pro-apoptotic and membrane-disruptive effects. These findings reinforce *Cabozantinib's* role as a selective anti-angiogenic therapy with favorable cell-sparing properties, suitable for long-term management of RCC.

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