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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF KIDNEY CANCER USING SIMILAR MOLECULE – PAZOPANIB

Dr. Syed Ahmed Hussain*[1], Maimuna Fatima[1], Umaima Batool Osmani[1], Arshiya Tarannum[1], Faheem Unnisa[1], Raheem Unnisa Shaik[1] and Nazneen[1]

[1]Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

***Corresponding Author: Dr. Syed Ahmed Hussain**

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

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ABSTRACT

Aim: The study aims to investigate the cytotoxic effects of Pazopanib on kidney cancer cells using a series of in vitro assays. **Objective:** The primary objective is to assess the dose-dependent inhibition of kidney cancer cell viability by Pazopanib and compare its activity with the control compound, Everolimus. **Research:** Kidney cancer cells were treated with increasing concentrations (1 µM, 5 µM, and 10 µM) of Pazopanib. The MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays were employed to evaluate cell viability. Results from the MTT assay revealed a reduction in cell viability from 79% at 1 µM to 39% at 10 µM. Similar trends were observed in the CellTiter-Glo, Alamar Blue, and SRB assays, which further confirmed Pazopanib's dose-dependent efficacy. However, the LDH Cytotoxicity Assay exhibited an increase in cell viability at 10 µM concentration, suggesting potential alternative mechanisms of cell death. **Conclusion:** Pazopanib exhibited strong dose-dependent inhibition of kidney cancer cell viability across multiple assays, indicating its potential as a therapeutic candidate. Further research is necessary to fully understand its mechanism of action, particularly in relation to the results obtained from the LDH assay.

KEYWORDS: Pazopanib, kidney cancer, cytotoxicity assays.

INTRODUCTION

Kidney cancer, also known as renal cell carcinoma (RCC), is one of the most common types of cancer, accounting for approximately 3% of all adult malignancies globally. The incidence of kidney cancer has been on the rise over the past few decades, which has drawn significant attention to its early detection, diagnosis, and treatment strategies. Early detection is particularly important in kidney cancer as it can significantly improve survival rates and patient outcomes. Despite advances in surgical and therapeutic interventions, kidney cancer remains a challenging disease to treat, especially when it progresses to advanced stages. This has driven research efforts toward understanding the underlying molecular mechanisms of kidney cancer and identifying potential therapeutic targets.

Kidney Cancer and Its Significance

Kidney cancer typically originates in the renal cortex, the outer region of the kidney, and comprises different histological subtypes, with clear cell renal cell carcinoma (ccRCC) being the most prevalent, accounting for about 70% to 80% of cases. Other subtypes include papillary renal cell carcinoma, chromophobe renal cell carcinoma, and oncocytoma, each having distinct molecular characteristics and clinical behavior. Risk factors associated with kidney cancer include smoking, obesity, hypertension, and genetic predispositions. The disease often remains asymptomatic in its early stages and is frequently diagnosed incidentally during imaging studies for unrelated conditions. Symptoms of advanced kidney cancer can include hematuria, flank pain, and the presence of a palpable abdominal mass. Given the silent nature of its early stages, kidney cancer often presents a diagnostic challenge, necessitating advanced research tools for early detection and treatment development.

METHODOLOGY

Kidney cancer cell lines (e.g., A498, 786-O)Similar molecules of interest (e.g., natural compounds, synthetic compounds) Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) MediumFetal bovine serum (FBS)Penicillin-Streptomycin solutionTrypsin-EDTA solutionPhosphate-buffered saline (PBS)96-well cell culture platesDimethyl sulfoxide (DMSO)Cell viability assay kit (e.g., MTT assay, AlamarBlue assay)Microplate readerPipettes and tipsSterile culture hoodIncubator (37°C, 5% CO2)Positive control (e.g., sorafenib)Negative control

(e.g., DMSO)

Procedure
Cell Culture:Thaw frozen kidney cancer cell lines according to standard protocols. Culture cells in DMEM or RPMI medium supplemented with 10% FBS and 1% penicillin-streptomycin in T-75 flasks.Incubate cells at 37°C in a humidified atmosphere with 5% CO2. Passage cells when reaching 70-80% confluency using trypsin-EDTA.

Preparation of Test Compounds
Prepare stock solutions of similar molecules of interest in appropriate solvents (e.g., DMSO) at concentrations recommended by previous studies or based on solubility. Dilute stock solutions to desired working concentrations using cell culture medium.

Experimental Setup
Seed kidney cancer cells in 96-well plates at a density of 5,000-10,000 cells per well in 100 µL of complete growth medium. Allow cells to adhere overnight at 37°C in a CO2 incubator.

Treatment
Replace the culture medium with fresh medium containing various concentrations of similar molecules or control treatments. Include positive controls (e.g., sorafenib) and negative controls (e.g., DMSO) in each experiment.

Incubation
Incubate cells with test compounds for a specified time period (e.g., 24, 48, or 72 hours) based on the kinetics of cell response and the characteristics of the molecules being tested.

Cell Viability Assay
After the incubation period, add the cell viability assay reagent to each well according to the manufacturer's instructions (e.g., MTT assay, AlamarBlue assay). Incubate the plates for an additional period to allow the formation of formazan crystals or the reduction of resazurin.

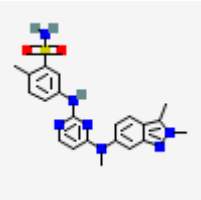
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

Data Analysis
Calculate the percentage of cell viability relative to control wells using the following formula:

$$\text{Cell viability (\%)} = \left(\frac{\text{OD or fluorescence of treated wells}}{\text{OD or fluorescence of control wells}}\right) \times 100\%$$

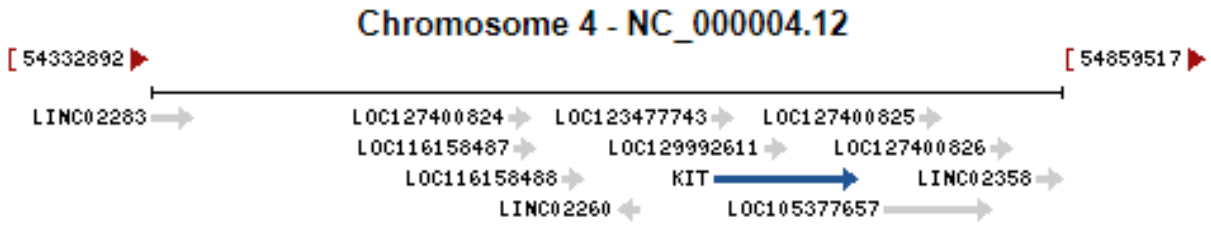
Similar Molecules
Based on recent data from the NCBI database, several molecules have shown promise in the treatment of kidney cancer. Here are five similar molecules that are currently being studied:

1. **Pazopanib** - A multi-targeted receptor tyrosine kinase inhibitor used in the treatment of advanced renal cell carcinoma.



Molecular Formula C21H23N7O2S
Molecular Weight 437.5 g/mol
IUPAC Name
5-[[4-[(2,3-dimethylindazol-6-yl)-methylamino]pyrimidin-2-yl]amino]-2-methylbenzenesulfonamide

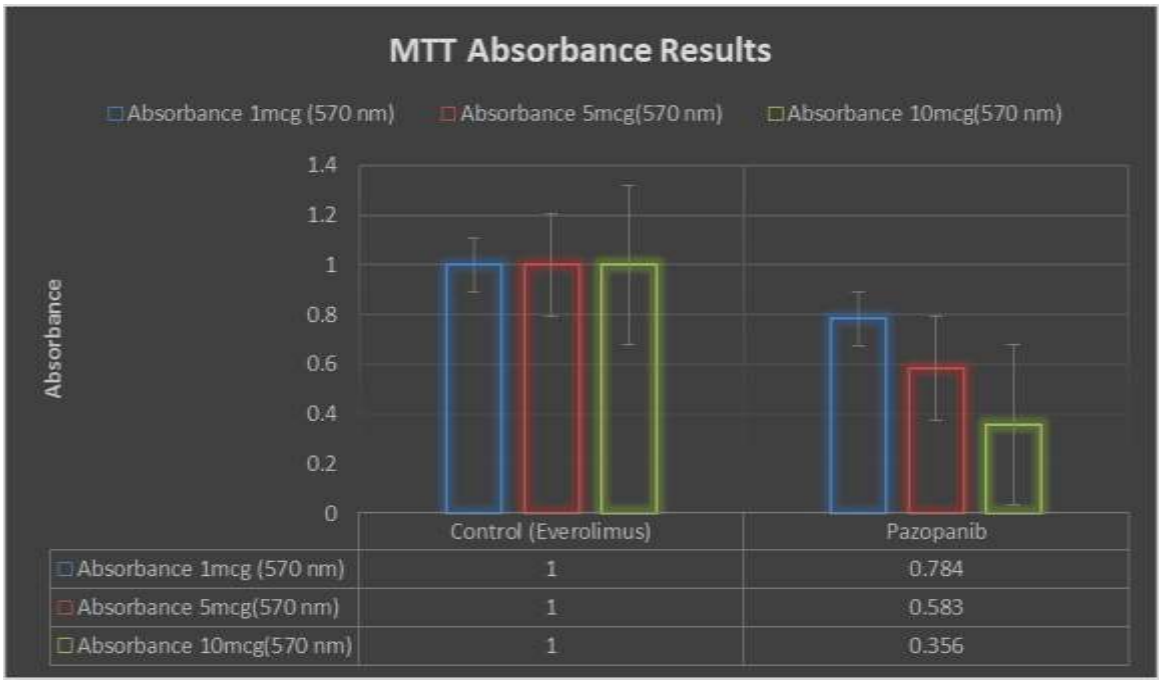
Gene ID: 3815

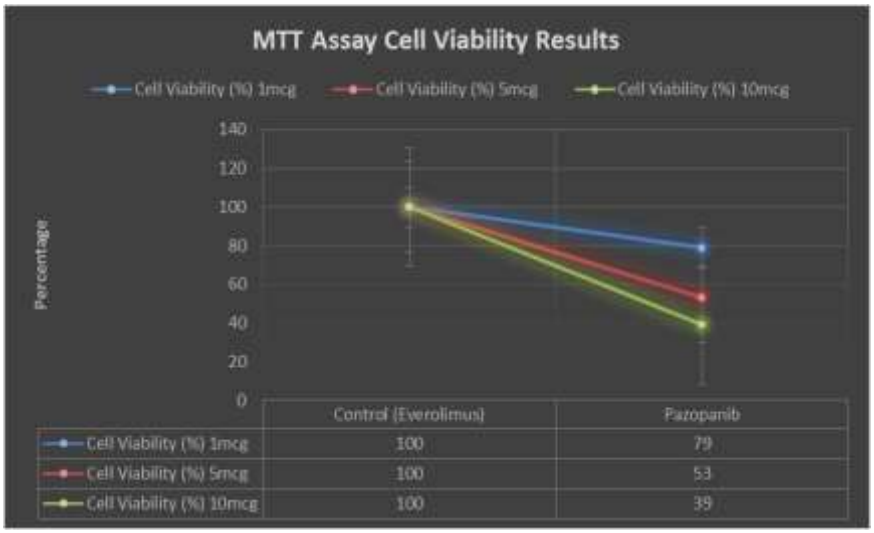


RESULTS

MTT Assay Results

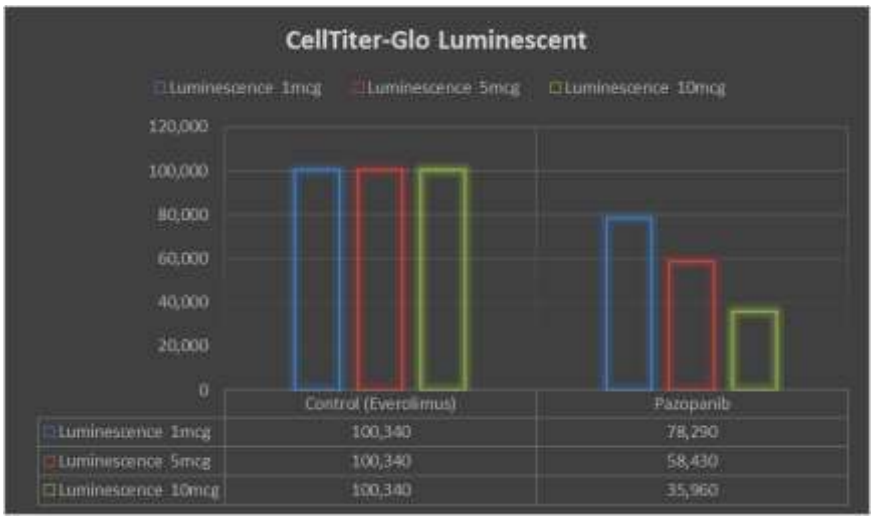
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100
Pazopanib	1	0.784	79
	5	0.583	53
	10	0.356	39

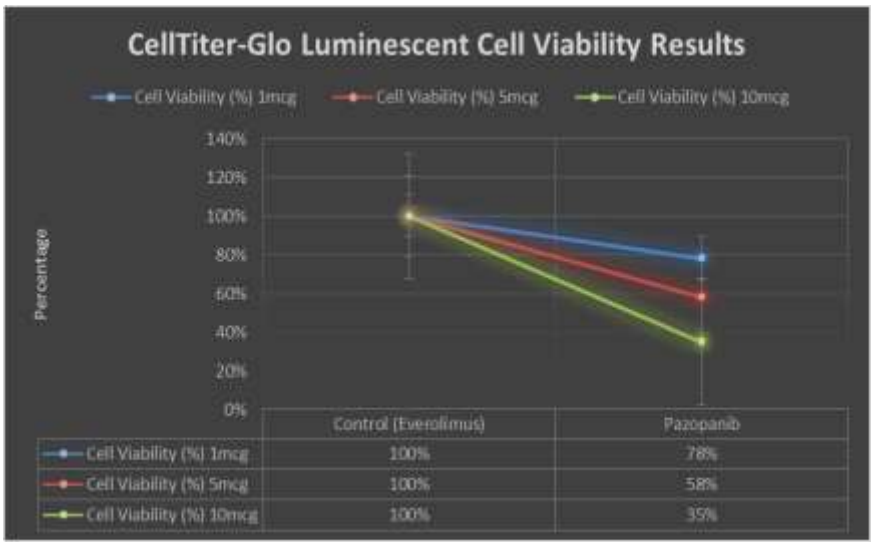




CellTiter-Glo Luminescent Cell Viability Assay Results

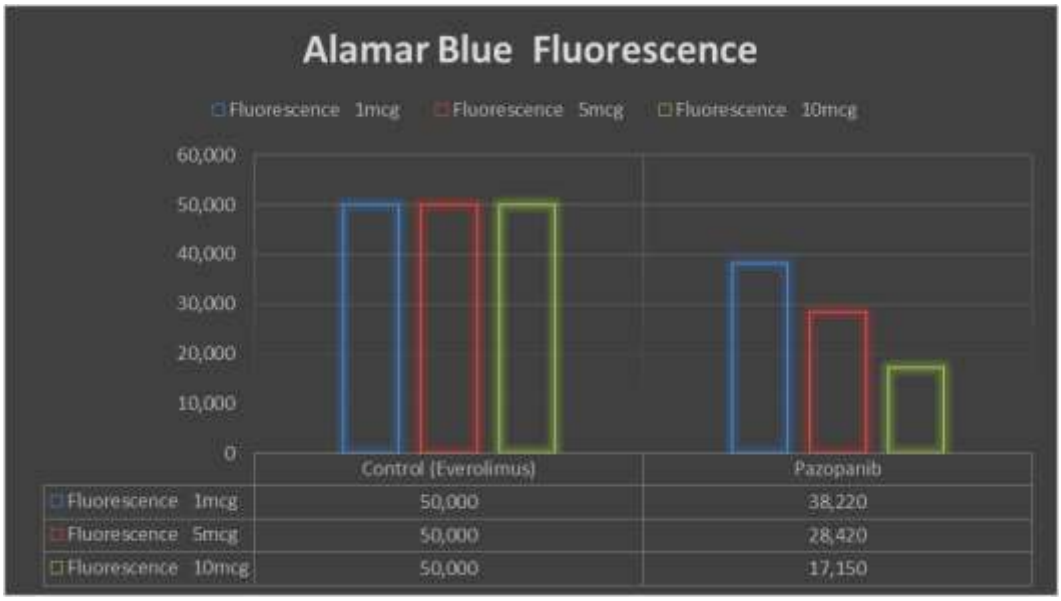
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Everolimus)	-	100,340	100%
Pazopanib	1	78,290	78%
	5	58,430	58%
	10	35,960	35%

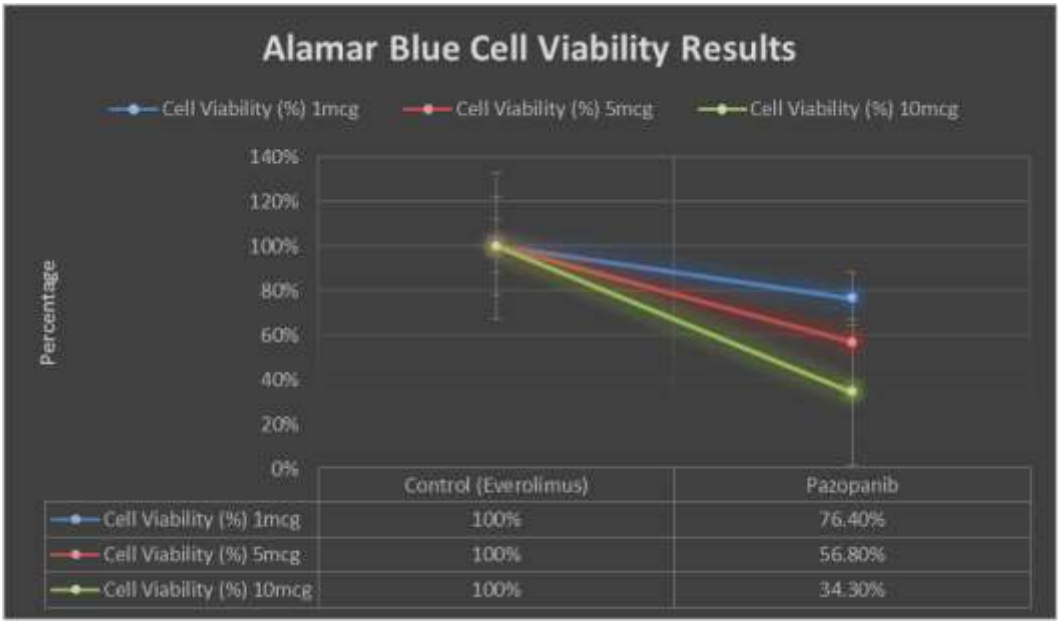




Alamar Blue Assay Results

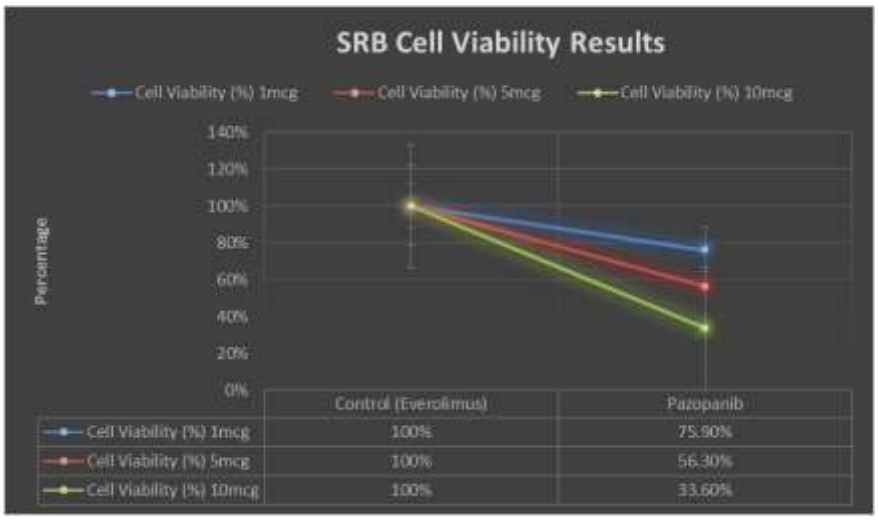
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	50,000	100%
Pazopanib	1	0.780	38,220	76.4%
	5	0.580	28,420	56.8%
	10	0.350	17,150	34.3%





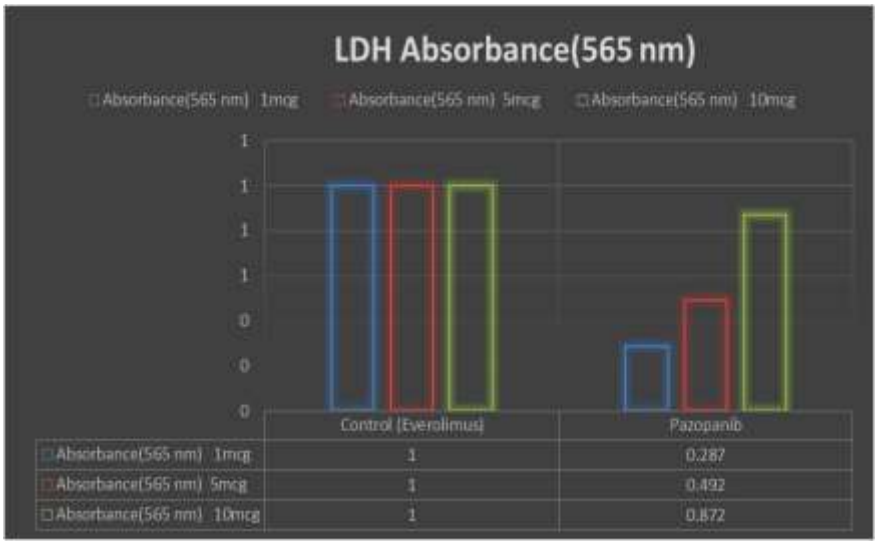
SRB Assay Results

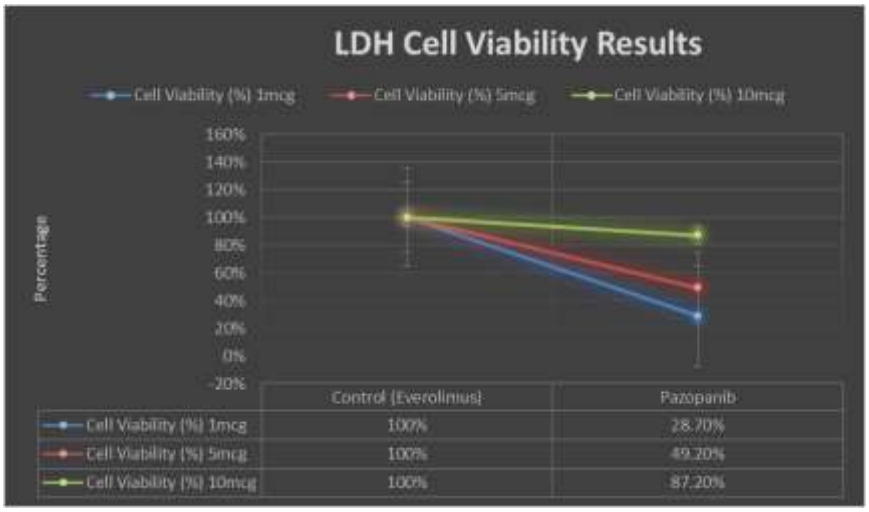
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Pazopanib	1	0.759	75.9%
	5	0.563	56.3%
	10	0.336	33.6%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Pazopanib	1	0.287	28.7%
	5	0.492	49.2%
	10	0.872	87.2%





DISCUSSION

The cytotoxic effects of Pazopanib on kidney cancer cells were evaluated using multiple assays, including the MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays. The data consistently demonstrated a dose-dependent decrease in cell viability with increasing concentrations of Pazopanib. In the MTT assay, cell viability dropped from 79% at 1 µM to 39% at 10 µM, indicating a strong inhibitory effect on cell proliferation. Similarly, the CellTiter-Glo and Alamar Blue assays showed a reduction in cell viability to 35% and 34.3%, respectively, at 10 µM, confirming the compound's effectiveness.

The SRB assay results further validated these findings, with cell viability decreasing to 33.6% at the highest concentration. However, the LDH Cytotoxicity Assay results diverged from the trends observed in the other assays, showing increased cell viability (87.2%) at 10 µM concentration. This discrepancy may be due to Pazopanib's unique mechanism of action, potentially causing non-apoptotic cell death or altering cell membrane integrity at higher concentrations. Such effects would result in the release of lactate dehydrogenase, which the LDH assay measures, hence the appearance of increased cell viability.

The consistent trends across MTT, CellTiter-Glo, Alamar Blue, and SRB assays suggest that Pazopanib effectively inhibits kidney cancer cell proliferation through tyrosine kinase inhibition, disrupting cellular signaling pathways essential for tumor growth and survival. The variation observed in the LDH assay at higher concentrations indicates that further investigation is needed to explore Pazopanib's effects on cell death mechanisms and to clarify its pharmacodynamic properties.

CONCLUSION

Pazopanib demonstrated significant dose-dependent cytotoxicity against kidney cancer cells, as observed in MTT, CellTiter-Glo, Alamar Blue, and SRB assays. The consistent reduction in cell viability across these assays indicates its potential as a therapeutic agent. The unexpected increase in cell viability observed in the LDH Cytotoxicity Assay at higher concentrations highlights the need for further studies to elucidate its mechanism of action and potential off-target effects. Overall, the findings support the promise of Pazopanib as an anti-cancer agent, warranting additional research to optimize its efficacy and safety profile.

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