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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF KIDNEY CANCER USING SIMILAR MOLECULE - AXITINIB AMIDE

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ABSTRACT

Aim: This study aims to evaluate the cytotoxic effects of Axitinib Amide on kidney cancer cells using multiple in vitro assays. **Objective:** The primary objective is to assess the dose-dependent inhibition of kidney cancer cell viability by Axitinib Amide and to compare its activity against the control compound, Everolimus. **Research:** Kidney cancer cells were treated with increasing concentrations (1 µM, 5 µM, and 10 µM) of Axitinib Amide. The assays performed include MTT, CellTiter-Glo Luminescent Cell Viability, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity. The results demonstrated a consistent reduction in cell viability across MTT, CellTiter-Glo, Alamar Blue, and SRB assays, indicating a strong dose-dependent cytotoxic effect. However, the LDH Cytotoxicity Assay showed an increase in cell viability at higher concentrations, suggesting potential alternate mechanisms of action or off-target effects. **Conclusion:** Axitinib Amide exhibited potent dose-dependent cytotoxicity against kidney cancer cells in multiple assays. Although the results were generally consistent, the divergent findings in the LDH Cytotoxicity Assay highlight the need for further research to understand its full pharmacological profile and mechanism of action.

KEYWORDS: Axitinib Amide, 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.

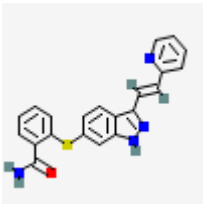
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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

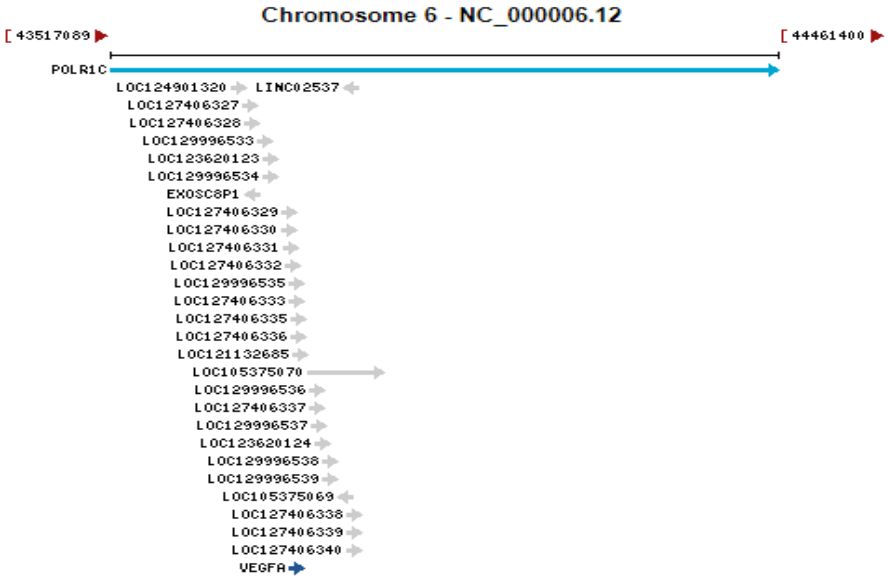
$$\text{Cell viability (\%)} = \left(\frac{\text{OD or fluorescence of treated wells}}{\text{OD or fluorescence of control wells}}\right) \times 100\%$$

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. **Axitinib Amide** - Another tyrosine kinase inhibitor that has demonstrated efficacy in treating renal cell carcinoma.



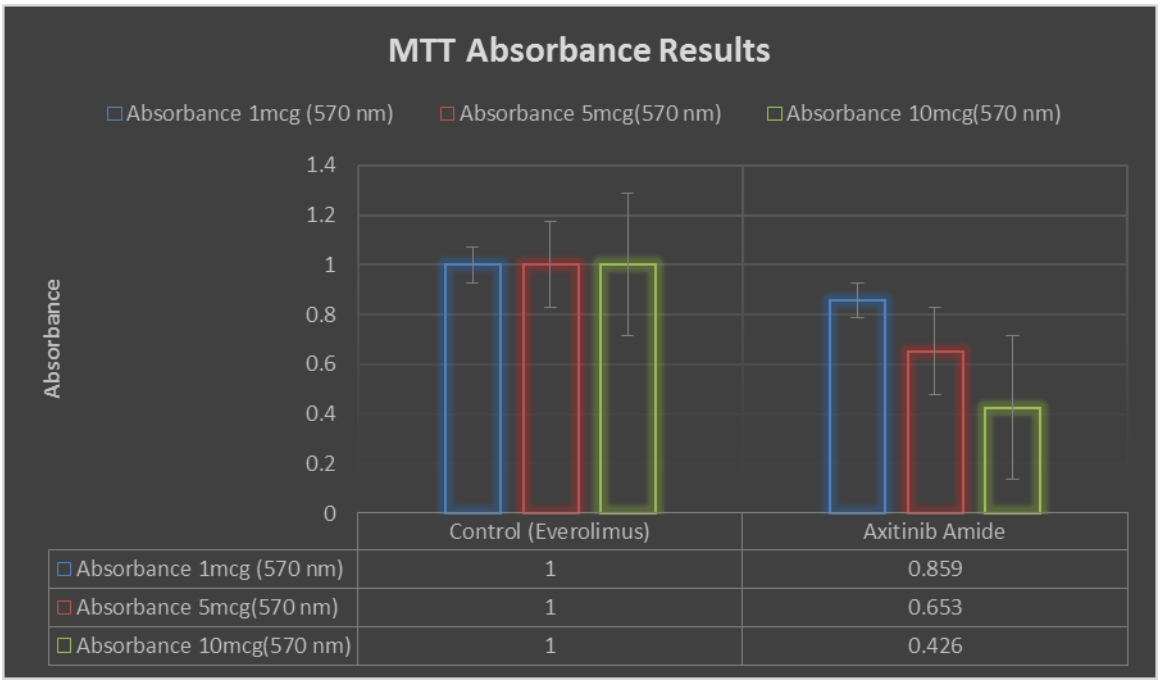
Molecular Formula C21H16N4OS
Molecular Weight 372.4 g/mol
IUPAC Name
Gene ID: 7422

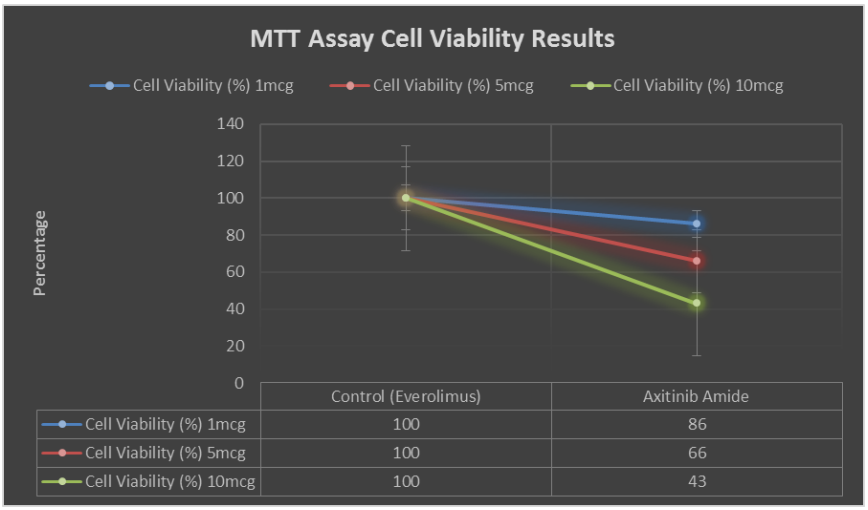


RESULTS

MTT Assay Results

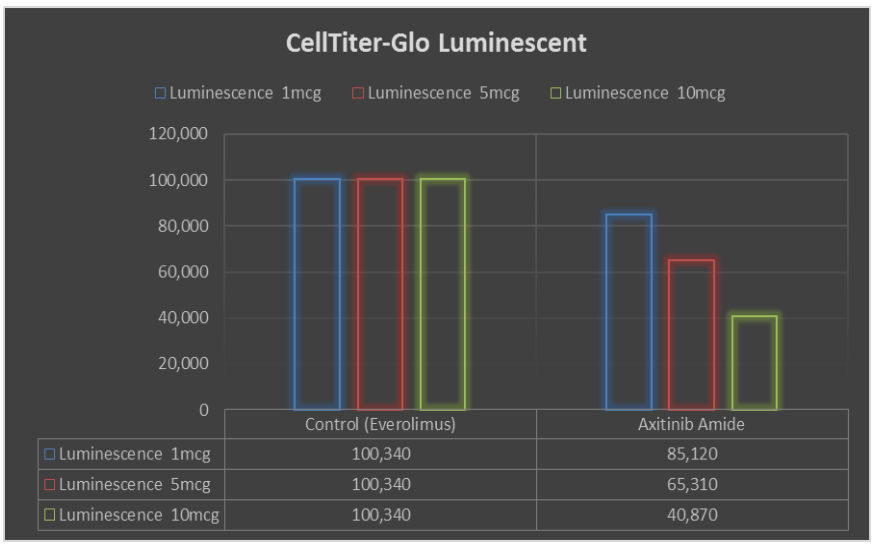
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100
Axitinib Amide	1	0.859	86
	5	0.653	66
	10	0.426	43

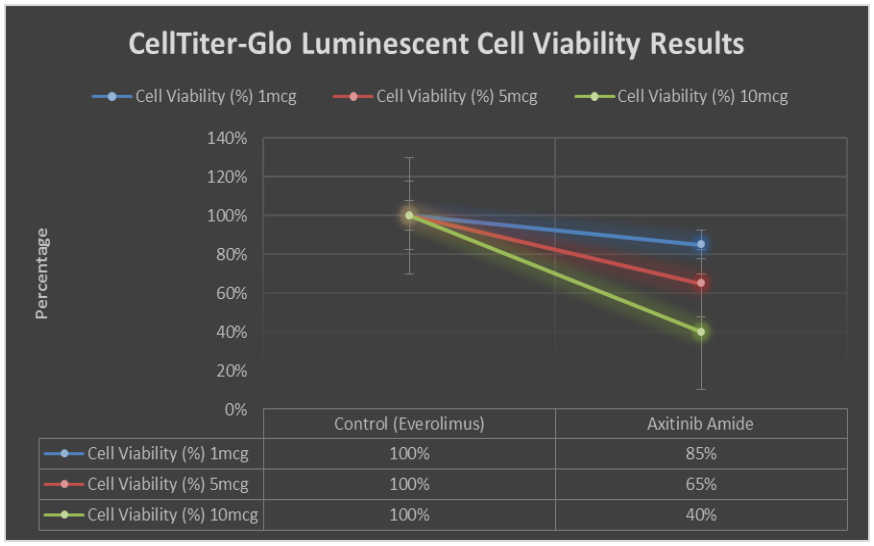




CellTiter-Glo Luminescent Cell Viability Assay Results

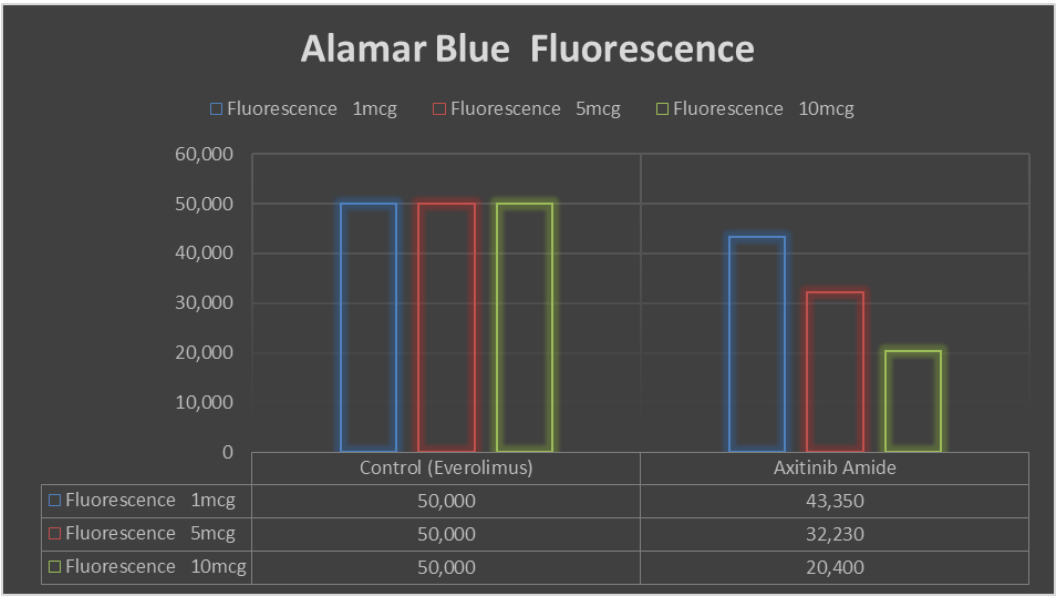
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Everolimus)	-	100,340	100%
Axitinib Amide	1	85,120	85%
	5	65,310	65%
	10	40,870	40%

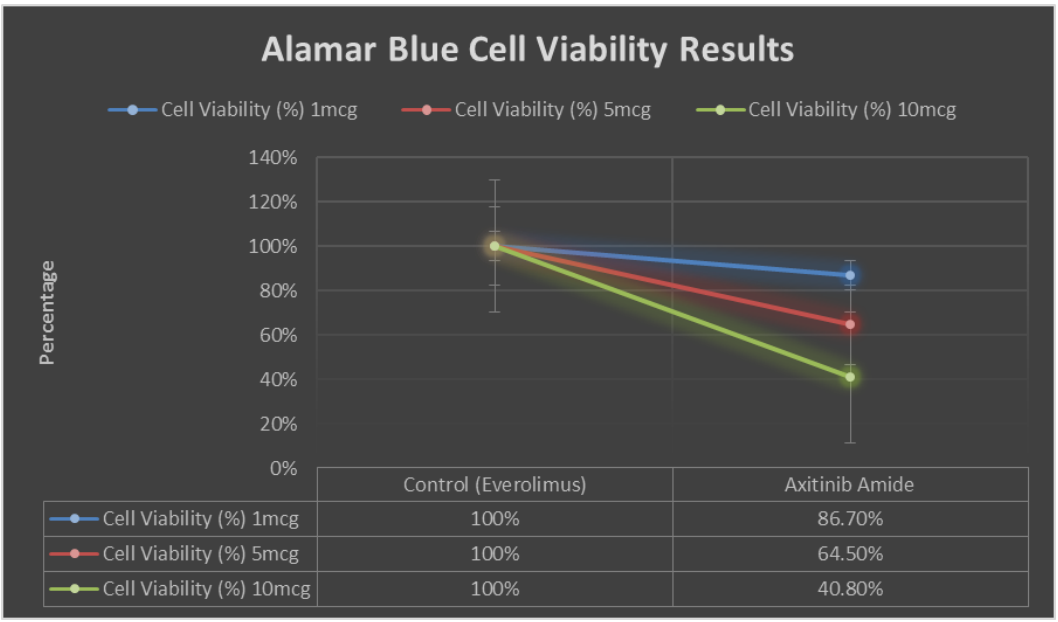




Alamar Blue Assay Results

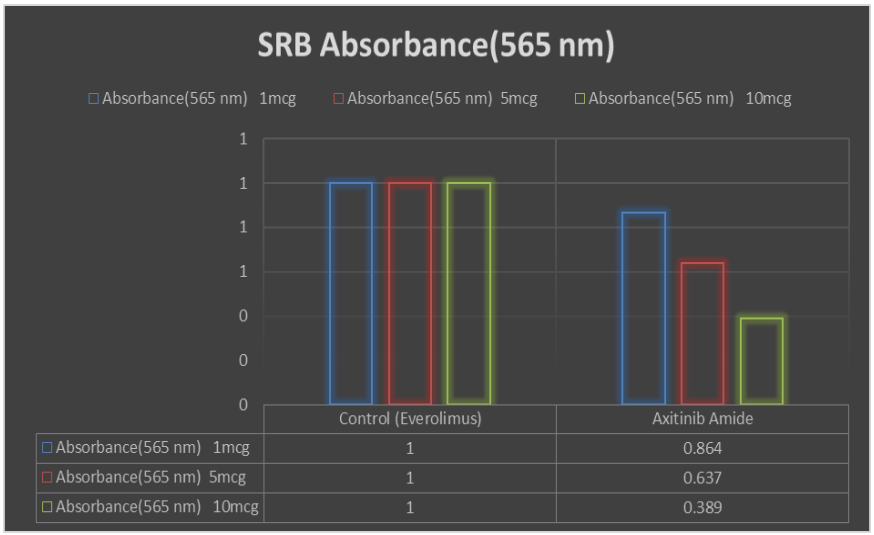
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	50,000	100%
Axitinib Amide	1	0.850	43,350	86.7%
	5	0.650	32,230	64.5%
	10	0.400	20,400	40.8%

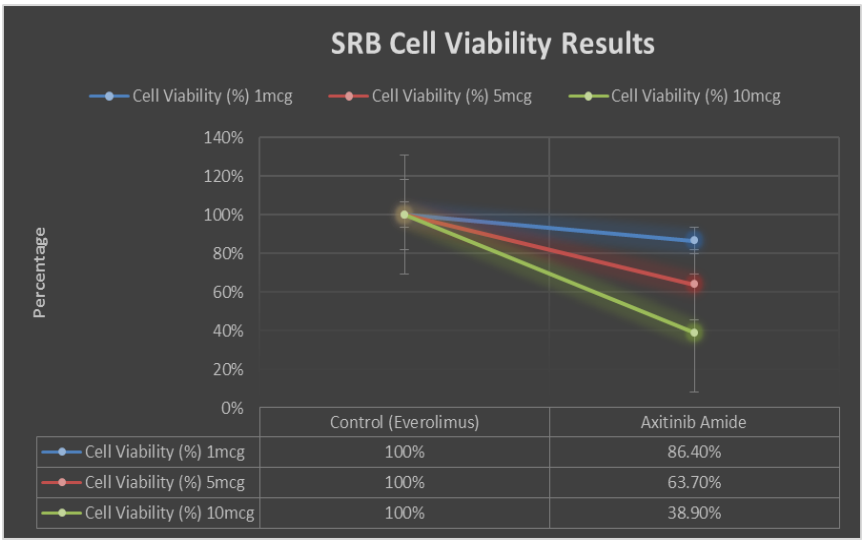




SRB Assay Results

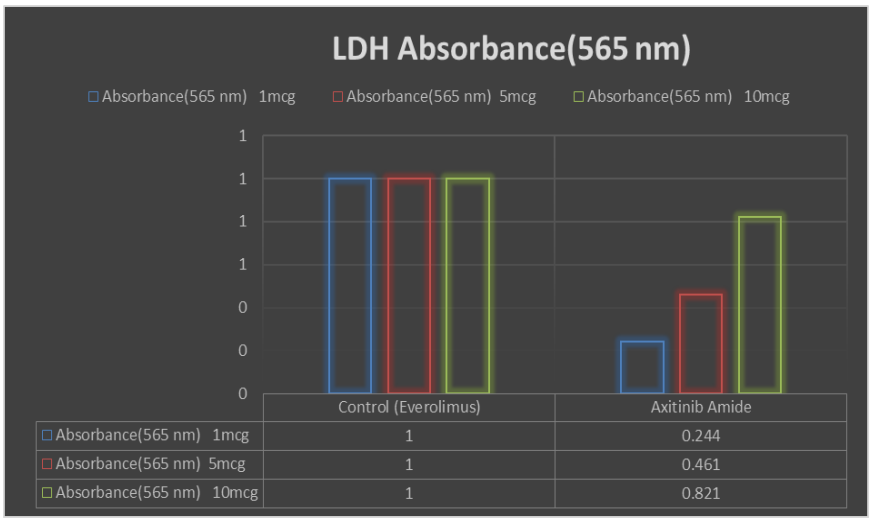
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Axitinib Amide	1	0.864	86.4%
	5	0.637	63.7%
	10	0.389	38.9%

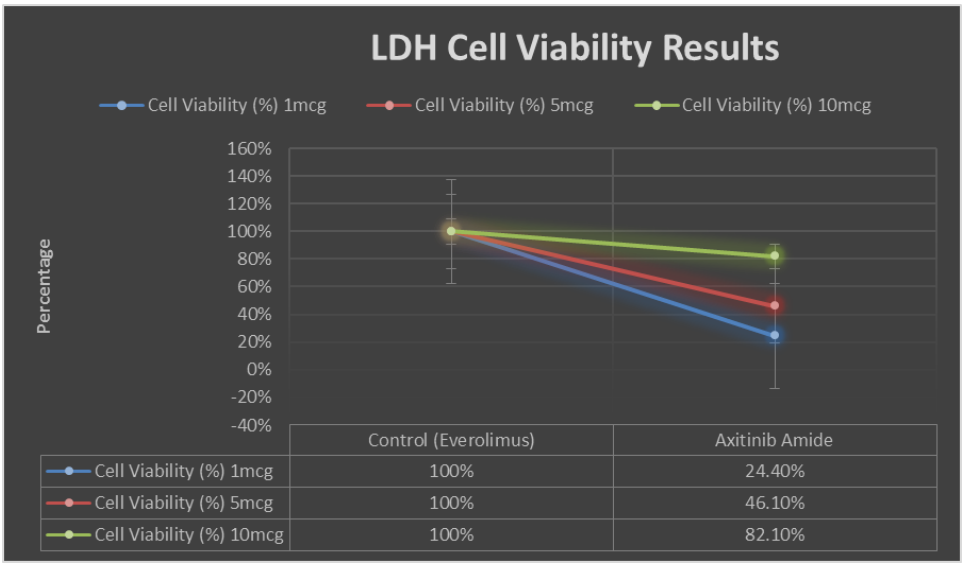




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Axitinib Amide	1	0.244	24.4%
	5	0.461	46.1%
	10	0.821	82.1%





DISCUSSION

The experimental results across multiple assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays, suggest that Axitinib Amide has a considerable dose-dependent cytotoxic effect on kidney cancer cells. In the MTT assay, cell viability decreased from 86% at 1 µM concentration to 43% at 10 µM concentration. Similar trends were observed in the CellTiter-Glo and Alamar Blue assays, where cell viability decreased to 40% and 40.8%, respectively, at the highest concentration of 10 µM.

The LDH Cytotoxicity Assay, however, displayed an unexpected increase in cell viability (82.1%) at 10 µM concentration, which diverged from the results of other assays. This anomaly might indicate that Axitinib Amide causes membrane disruption or induces a necrotic form of cell death that the LDH assay is sensitive to, or it could suggest the occurrence of off-target effects at higher concentrations. The relatively high cell viability observed in the LDH assay at 10 µM could potentially reflect a cytostatic rather than cytotoxic mechanism, implying that Axitinib Amide may inhibit cell proliferation without immediately causing cell death at higher doses.

Overall, the consistency in the decrease of cell viability across most assays indicates that Axitinib Amide has potential as a therapeutic agent for kidney cancer. The divergence observed in the LDH results suggests a need for further studies to investigate the exact mechanisms involved, especially at higher concentrations.

CONCLUSION

Axitinib Amide showed a strong dose-dependent cytotoxic effect on kidney cancer cells, as evidenced by consistent reductions in cell viability in the MTT, CellTiter-Glo, Alamar Blue, and SRB assays. The unexpected trend observed in the LDH Cytotoxicity Assay at higher concentrations indicates the potential for alternate mechanisms of action or off-target effects that warrant further investigation. These findings suggest that Axitinib Amide has therapeutic potential for kidney cancer treatment, but a deeper understanding of its pharmacological profile is needed to confirm its efficacy and safety.

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