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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF KIDNEY CANCER USING SIMILAR MOLECULE - 5-METHOXY-SUNITINIB

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ABSTRACT

Aim: The study aims to evaluate the efficacy of 5-Methoxy-sunitinib in reducing kidney cancer cell viability using various cytotoxicity and viability assays. **Objective:** The primary objective is to assess the dose-dependent cytotoxic effects of 5-Methoxy-sunitinib on kidney cancer cells and to compare its effectiveness to the control compound, Everolimus. **Research:** Kidney cancer cells were treated with different concentrations (1 µM, 5 µM, 10 µM) of 5-Methoxy-sunitinib. The assays used included the MTT, CellTiter-Glo Luminescent Cell Viability, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity assays. The results indicated a consistent decrease in cell viability with increasing concentrations of 5-Methoxy-sunitinib, suggesting a dose-dependent inhibition of cancer cell growth. While all assays showed similar trends, the LDH assay results suggested alternative pathways of cell death at higher concentrations, warranting further investigation. **Conclusion:** 5-Methoxy-sunitinib demonstrated a strong potential in inhibiting kidney cancer cell proliferation in a dose-dependent manner across multiple assays. These findings highlight its promise as a therapeutic candidate for kidney cancer, though additional research is needed to fully elucidate its mechanism of action and therapeutic profile.

KEYWORDS: 5-Methoxy-sunitinib, 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 solution Trypsin-EDTA solution Phosphate-buffered saline (PBS)96-well cell culture plates Dimethyl 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

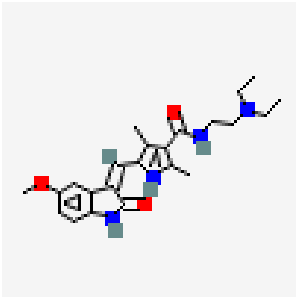
Calculate the percentage of cell viability relative to control wells using the following formula:

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

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. **5-Methoxy-sunitinib** - A tyrosine kinase inhibitor that targets multiple receptors involved in tumor growth and angiogenesis.

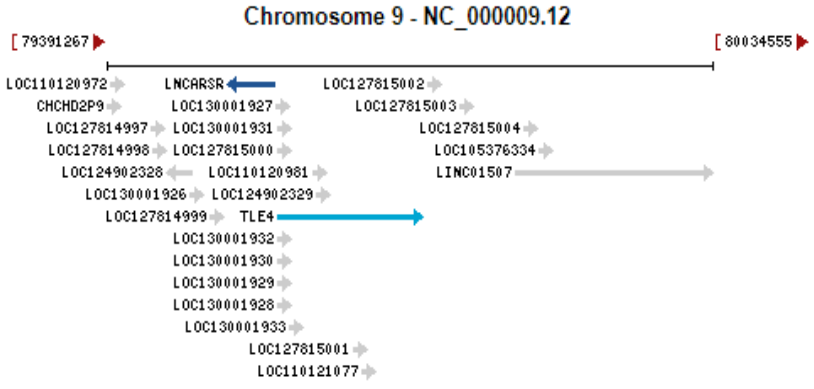


Molecular Formula C23H30N4O3
Molecular Weight 410.5 g/mol

IUPAC Name

N-[2-(diethylamino)ethyl]-5-[(Z)-(5-methoxy-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide

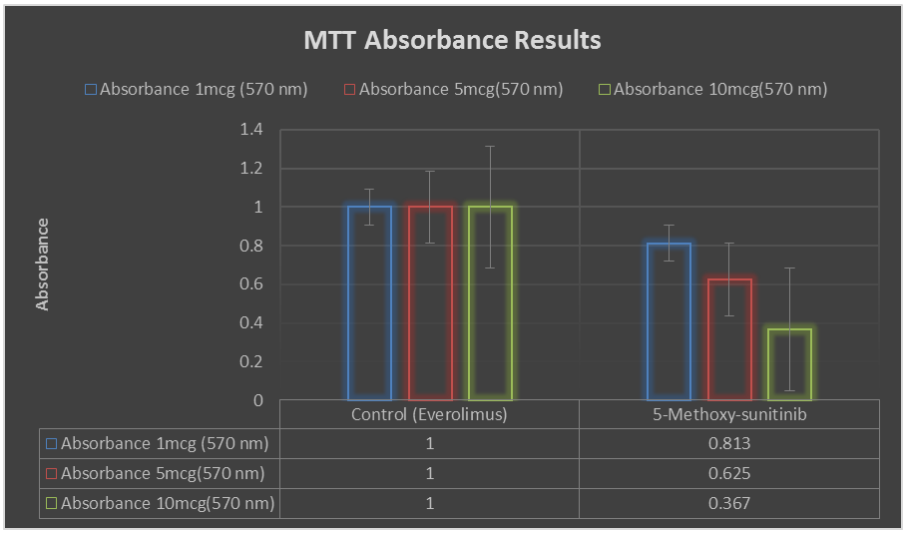
Gene ID: 102723932

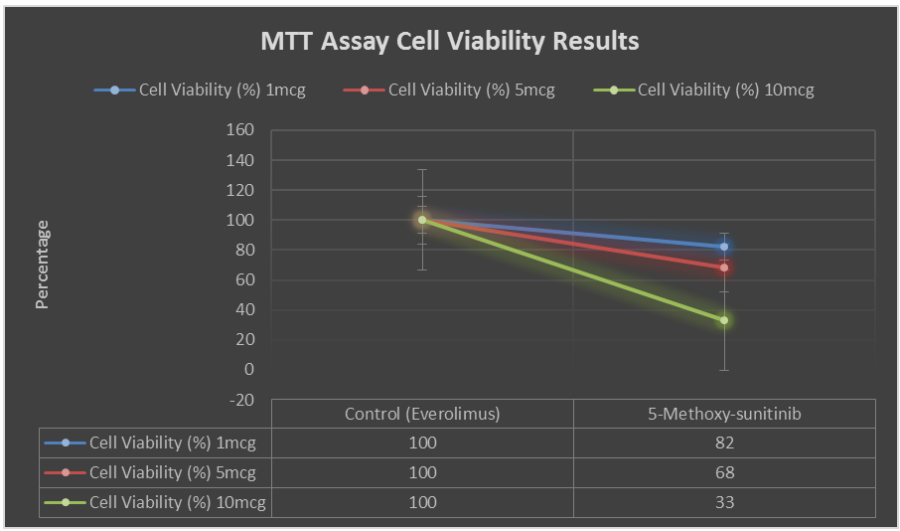


RESULTS

MTT Assay Results

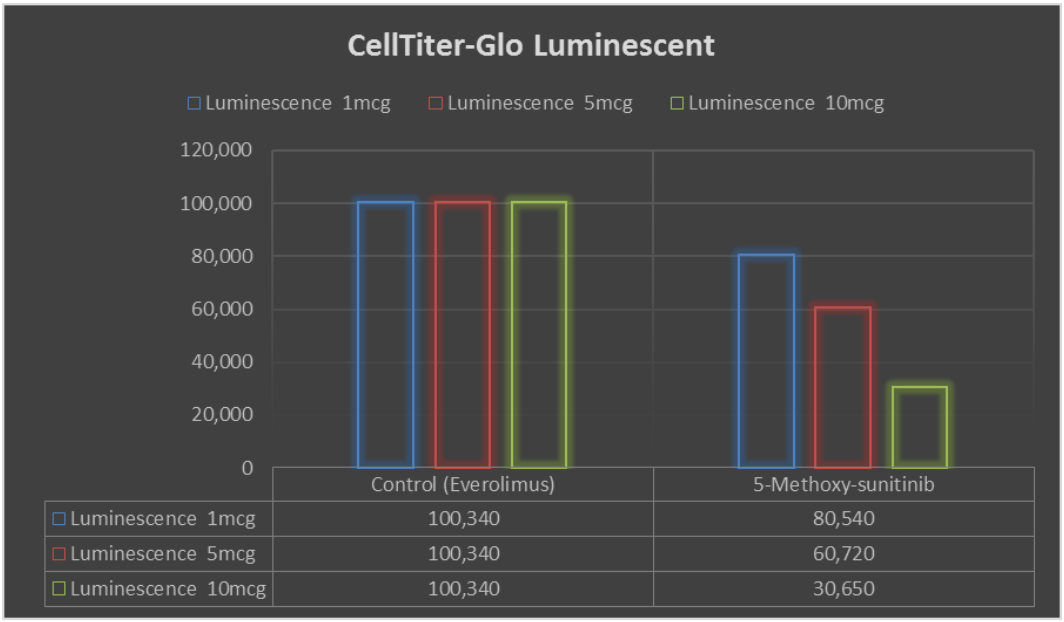
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100
5-Methoxy-sunitinib	1	0.813	82
	5	0.625	68
	10	0.367	33

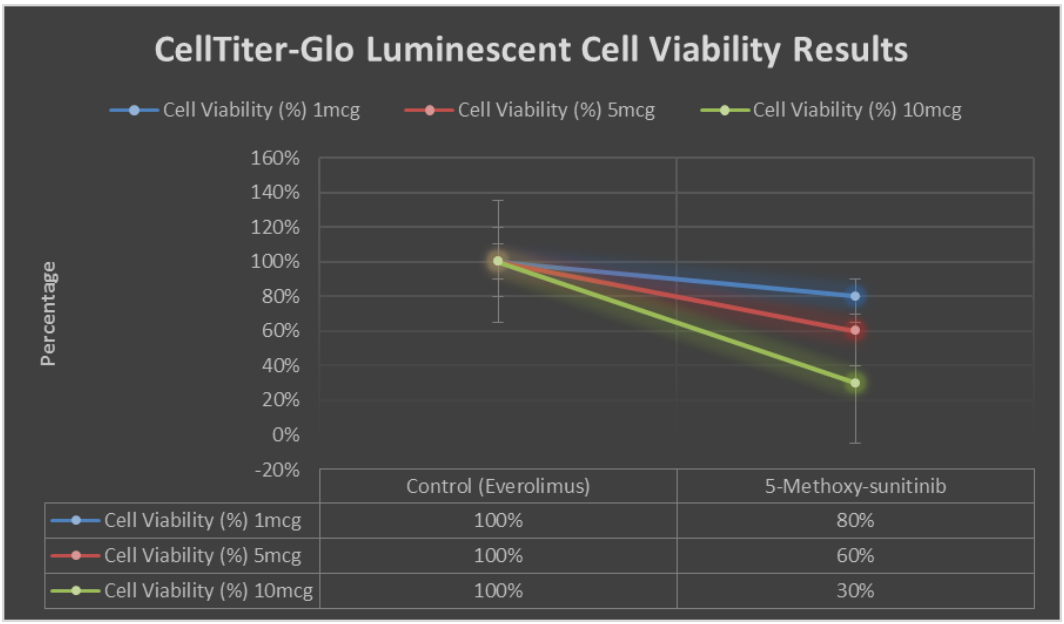




CellTiter-Glo Luminescent Cell Viability Assay Results

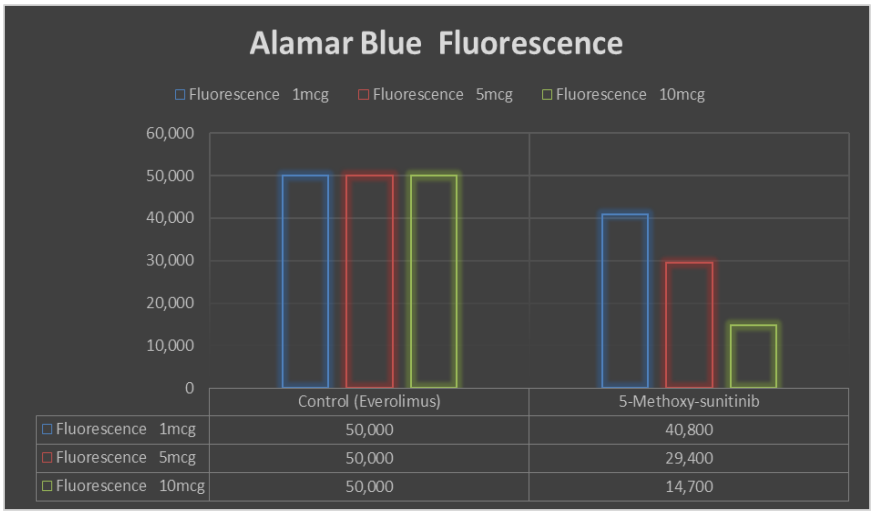
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Everolimus)	-	100,340	100%
5-Methoxy-sunitinib	1	80,540	80%
	5	60,720	60%
	10	30,650	30%

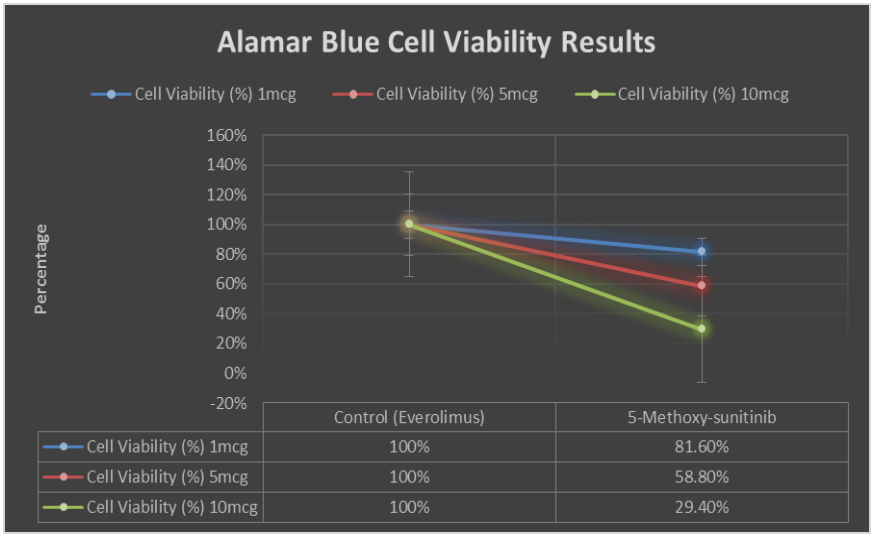




Alamar Blue Assay Results

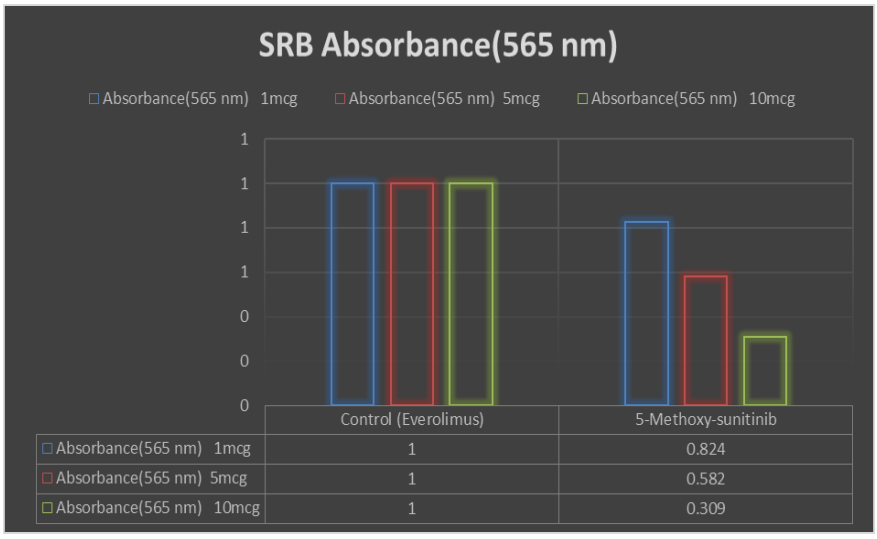
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	50,000	100%
5-Methoxy-sunitinib	1	0.800	40,800	81.6%
	5	0.600	29,400	58.8%
	10	0.300	14,700	29.4%

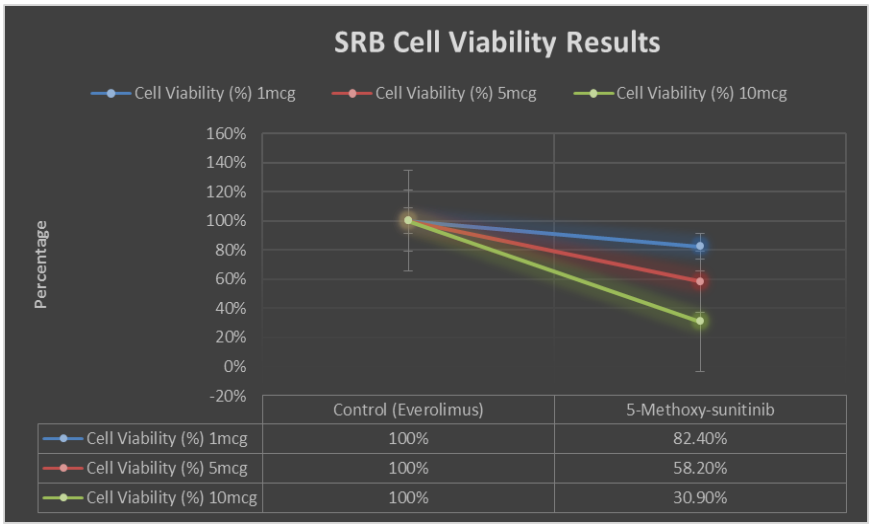




SRB Assay Results

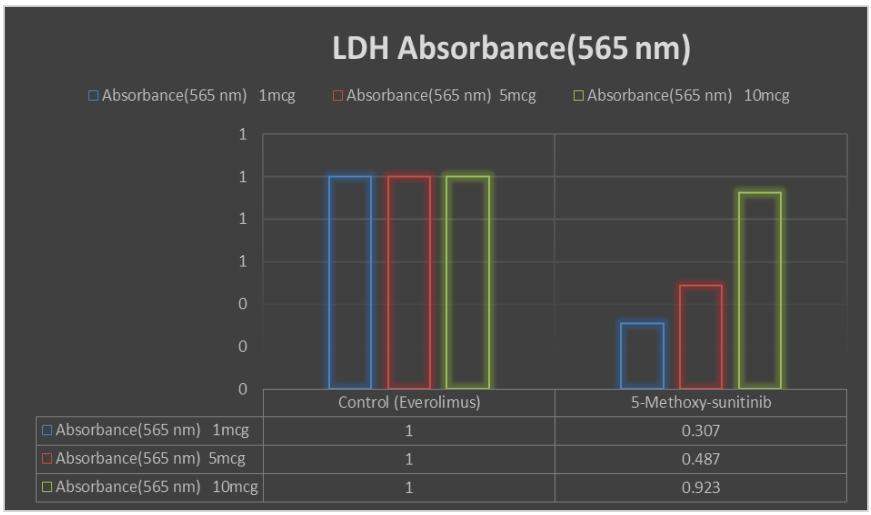
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
5-Methoxy-sunitinib	1	0.824	82.4%
	5	0.582	58.2%
	10	0.309	30.9%

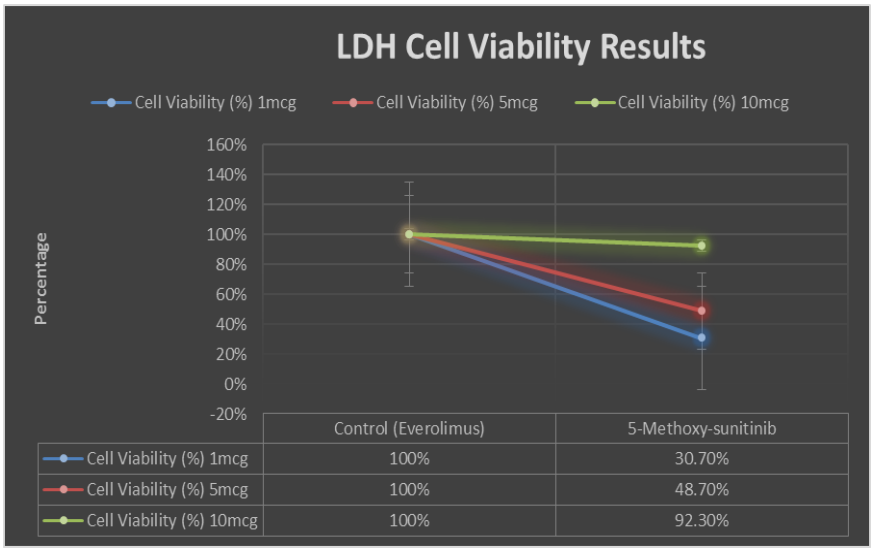




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
5-Methoxy-sunitinib	1	0.307	30.7%
	5	0.487	48.7%
	10	0.923	92.3%





DISCUSSION

The results from the various cytotoxicity and viability assays, namely the MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH assays, demonstrate a clear trend in the activity of 5-Methoxy-sunitinib against kidney cancer cells. Across all assays, it was observed that the cell viability decreased significantly as the concentration of 5-Methoxy-sunitinib increased. In the MTT assay, a 10 µM concentration resulted in a decrease in cell viability to 33%, while the CellTiter-Glo and Alamar Blue assays reported similar reductions to 30% and 29.4%, respectively. This consistent reduction in cell viability with increased concentration suggests that 5-Methoxy-sunitinib has a dose-dependent cytotoxic effect.

Interestingly, the LDH Cytotoxicity Assay showed an inverse trend, with the highest concentration of 5-Methoxy-sunitinib (10 µM) leading to 92.3% cell viability. This anomaly may indicate that at higher concentrations, the compound may induce cell membrane integrity disruption or an alternative pathway of cell death, such as necrosis, which the LDH assay is particularly sensitive to. Thus, it is crucial to interpret the LDH results in conjunction with other assays to obtain a comprehensive understanding of the cytotoxic effects of 5-Methoxy-sunitinib.

The overall findings suggest that 5-Methoxy-sunitinib is a potent inhibitor of cell proliferation in kidney cancer, with a dose-dependent efficacy. Its mechanism of action, likely through tyrosine kinase inhibition, effectively disrupts the signaling pathways essential for cell growth and angiogenesis. Further investigations are needed to elucidate its specific molecular targets and potential off-target effects.

CONCLUSION

5-Methoxy-sunitinib exhibits a strong dose-dependent inhibitory effect on kidney cancer cell viability across multiple assays, indicating its potential as an effective therapeutic agent. While the results are promising, the divergent results observed in the LDH Cytotoxicity Assay suggest the need for further investigation to fully understand the mechanism of action and its implications at varying concentrations. The overall consistency in the reduction of cell viability across assays like MTT, CellTiter-Glo, and Alamar Blue reinforces the potential of 5-Methoxy-sunitinib as a candidate for further development in cancer therapeutics.

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