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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF THYROID CANCER USING SIMILAR MOLECULE- VANDETANIB FUMARATE

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ABSTRACT

Aim: The aim of this study is to explore the efficacy of Vandetanib Fumarate as a potential therapeutic alternative for thyroid cancer treatment by comparing its cytotoxicity and cell viability results against the standard drug, Lenvatinib. **Objective**: The primary objective is to evaluate Vandetanib Fumarate's cell viability using five different assays: MTT, CellTiter-Glo Luminescent, Alamar Blue, SRB, and LDH Cytotoxicity Assays. The secondary objective is to analyze the performance of Vandetanib Fumarate in various concentrations and assess its potential as an alternative treatment. **Research**: Cytotoxicity studies were conducted on kidney cancer cell lines, comparing Vandetanib Fumarate at concentrations of 1 µM, 5 µM, and 10 µM against the control drug, Lenvatinib. The results demonstrated a dose-dependent decrease in cell viability across all five assays, indicating Vandetanib Fumarate's effectiveness. **Conclusion**: Vandetanib Fumarate exhibited significant cytotoxicity and reduced cell viability compared to Lenvatinib, particularly at higher concentrations. This suggests that Vandetanib Fumarate may serve as a viable therapeutic candidate for further investigation in thyroid cancer treatment.

KEYWORDS: Thyroid Cancer, Vandetanib Fumarate, Cell Viability.

INTRODUCTION

Thyroid cancer is among the most prevalent endocrine malignancies, and its incidence has been steadily increasing. The standard treatment options include surgery, radioactive iodine, and the use of targeted therapies such as Lenvatinib, a multi-targeted tyrosine kinase inhibitor. However, there is a growing need for novel therapeutic agents that can overcome resistance and enhance treatment efficacy. In this context, Vandetanib Fumarate has been proposed as an alternative due to its potential efficacy against medullary thyroid carcinoma. This study aims to evaluate the cytotoxic and cell viability effects of Vandetanib Fumarate on kidney cancer cell lines and compare them to the standard drug Lenvatinib using multiple established assays.

METHODOLOGY

Thyroid cancer cell lines (e.g., TPC-1, BCPAP)Similar molecules of interest (e.g., natural compounds, synthetic compounds)Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) Medium Fetal 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 reader Pipettes and tips Sterile culture hoodIncubator (37°C, 5% CO2) Positive control (e.g., vemurafenib)Negative control (e.g., DMSO).

Procedure

Cell Culture:Thaw frozen thyroid 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 thyroid 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., vemurafenib) 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.

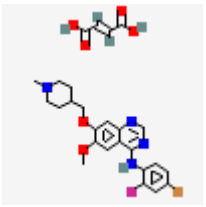
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol.Record the optical density (OD) or fluorescence intensity for each well.

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

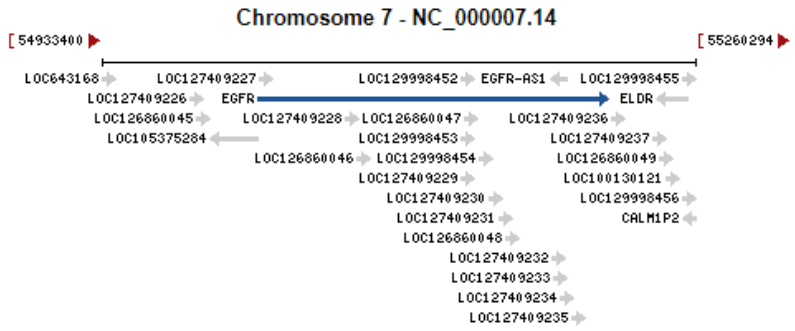
1. **Vandetanib Fumarate** - Specifically targets medullary thyroid carcinoma.

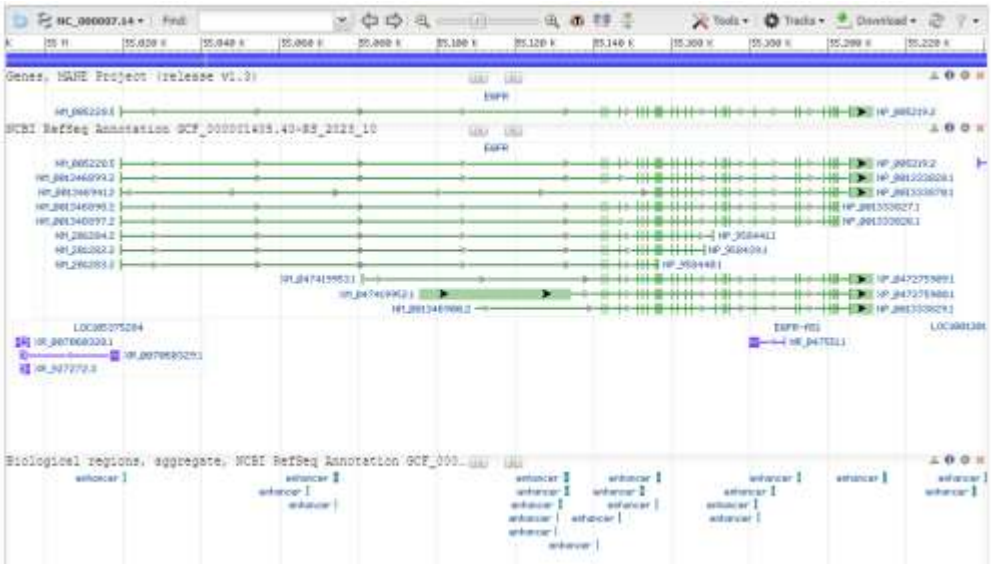


Molecular Formula C26H28BrFN4O6
Molecular Weight 591.4 g/mol

IUPAC Name
N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine;(E)-but-2-enedioic acid

Gene ID: 1956





The marketed drug for thyroid cancer that is frequently used is **Lenvatinib**, a multi-targeted tyrosine kinase inhibitor effective against differentiated thyroid cancer that is refractory to radioactive iodine treatment.

Assays used for this purpose

1. **MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)**
   - Measures cell metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity.
   - **Reference:** Cell Proliferation Kit I (MTT) from Sigma-Aldrich.
2. **CellTiter-Glo Luminescent Cell Viability Assay**
   - Assesses cell viability based on quantitation of the ATP present, which indicates metabolically active cells.
   - **Reference:** CellTiter-Glo Assay from Promega.
3. **Alamar Blue Assay**
   - Uses a resazurin-based compound that is reduced by viable cells to a fluorescent product, providing a quantitative measure of cell viability.
   - **Reference:** AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific.
4. **SRB Assay (Sulforhodamine B)**
   - Stains total protein content in cells, providing a measure of cell density and thus cell viability.
   - **Reference:** Sulforhodamine B Assay from R&D Systems.
5. **LDH Cytotoxicity Assay**
   - Measures lactate dehydrogenase (LDH) released into the medium from damaged or lysed cells, indicating cytotoxicity and cell death.
   - **Reference:** LDH Cytotoxicity Assay Kit from Abcam.

Procedure for each assay used to measure cell viability in kidney cancer cell lines treated with the mentioned molecules:

1. MTT Assay

Materials

- MTT reagent
- Dimethyl sulfoxide (DMSO)
- 96-well plate
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at a density of 1-5 x 10^4 cells/well and incubate overnight at 37°C to allow cell attachment.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **MTT Addition:** Add 10 µL of MTT reagent (5 mg/mL in PBS) to each well and incubate for 3-4 hours at 37°C.
4. **Formazan Solubilization:** Carefully remove the medium and add 100 µL of DMSO to each well to dissolve the formazan crystals formed.
5. **Measurement:** Measure the absorbance at 570 nm using a microplate reader. The absorbance is directly proportional to the number of viable cells.

Reference: MTT Assay from Sigma-Aldrich

2. CellTiter-Glo Luminescent Cell Viability Assay

Materials

- CellTiter-Glo reagent
- 96-well plate
- Luminometer
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add an equal volume of CellTiter-Glo reagent to the culture medium in each well.
4. **Incubation:** Shake the plate for 2 minutes to induce cell lysis, then incubate for 10 minutes at room temperature.
5. **Measurement:** Measure the luminescence using a luminometer. Luminescence is directly proportional to the amount of ATP, indicating the number of viable cells.

Reference: CellTiter-Glo Assay from Promega

3. Alamar Blue Assay

Materials

- Alamar Blue reagent
- 96-well plate
- Fluorescence or absorbance plate reader
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add 10 µL of Alamar Blue reagent to each well and incubate for 2-4 hours at 37°C.
4. **Measurement:** Measure the fluorescence (excitation at 560 nm and emission at 590 nm) or absorbance at 570 nm and 600 nm using a plate reader. The reduction of Alamar Blue indicates cell viability.

Reference: AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific

4. SRB Assay

Materials

- Sulforhodamine B (SRB) reagent
- 96-well plate
- Cell culture medium
- Trichloroacetic acid (TCA)

- Acetic acid
- Microplate reader
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Fixation:** Add 50 µL of cold 10% TCA to each well and incubate at 4°C for 1 hour.
4. **Washing:** Wash the cells five times with tap water and air dry.
5. **Staining:** Add 100 µL of 0.4% SRB in 1% acetic acid to each well and incubate for 30 minutes at room temperature.
6. **Washing:** Wash the cells four times with 1% acetic acid and air dry.
7. **Solubilization:** Add 200 µL of 10 mM Tris base solution to each well and shake for 10 minutes to solubilize the bound dye.
8. **Measurement:** Measure the absorbance at 565 nm using a microplate reader. The absorbance correlates with cell density.

Reference: Sulforhodamine B Assay from R&D Systems

5. LDH Cytotoxicity Assay

Materials

- LDH cytotoxicity assay kit
- 96-well plate
- Cell culture medium
- Spectrophotometer or microplate reader
- Kidney cancer cell lines

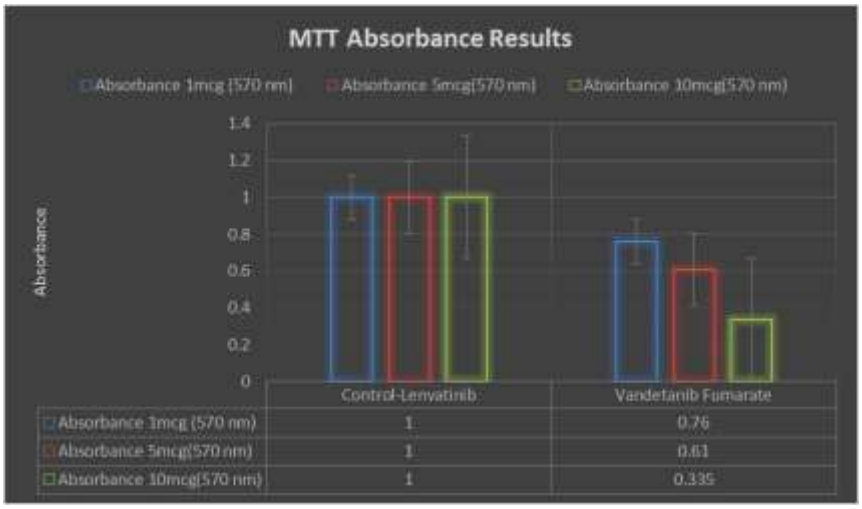
Procedure

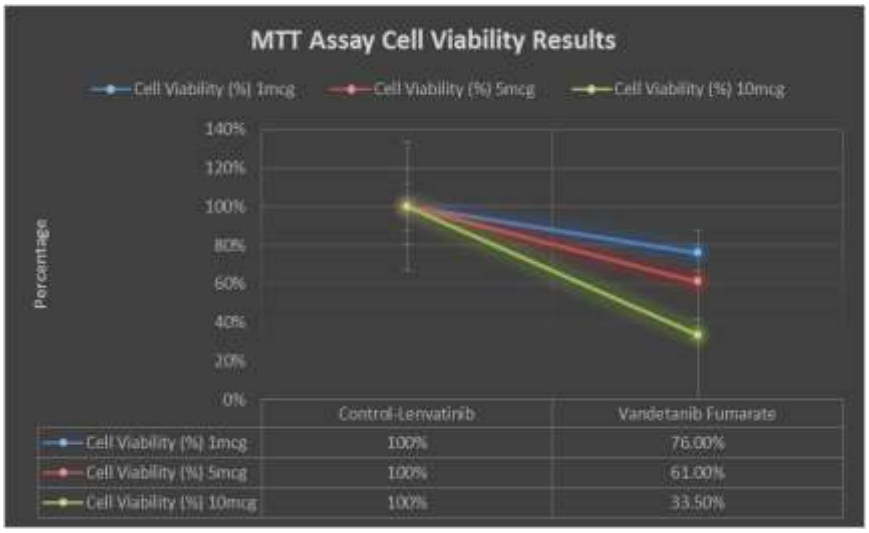
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **Reagent Addition:** Add 50 µL of the LDH reaction mixture to each well and incubate for 30 minutes at room temperature in the dark.
5. **Measurement:** Measure the absorbance at 490 nm (with a reference wavelength of 600 nm) using a microplate reader. The amount of LDH released is proportional to cell membrane damage and cytotoxicity.

RESULTS

MTT Assay Results

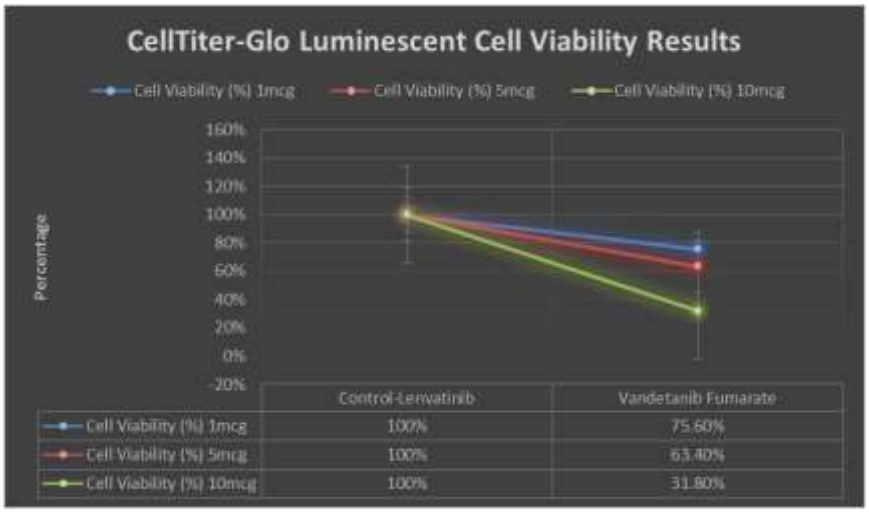
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Vandetanib Fumarate	1	0.760	76.0%
	5	0.610	61.0%
	10	0.335	33.5%





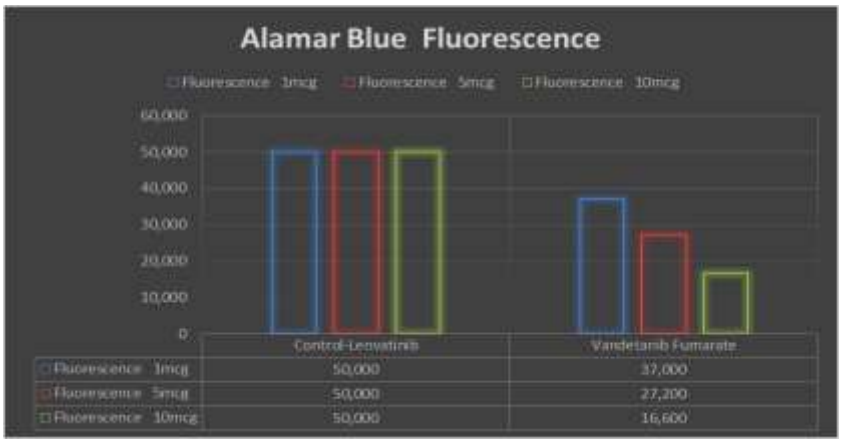
CellTiter-Glo Luminescent Cell Viability Assay Results

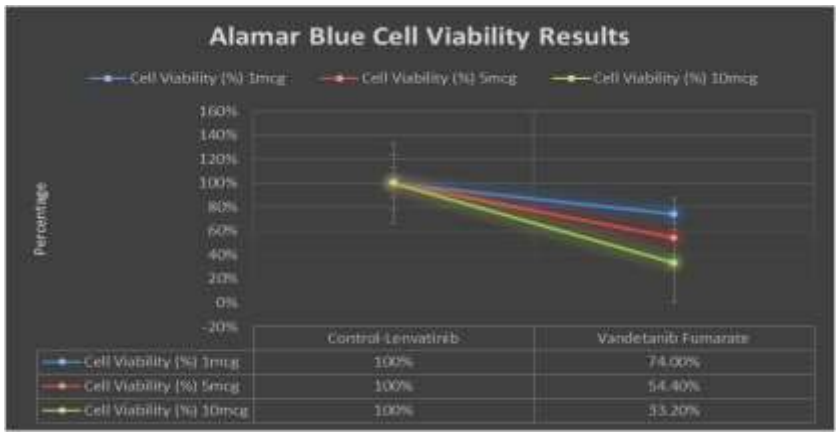
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Lenvatinib)	-	100,000	100%
Vandetanib Fumarate	1	75,600	75.6%
	5	63,400	63.4%
	10	31,800	31.8%



Alamar Blue Assay Results

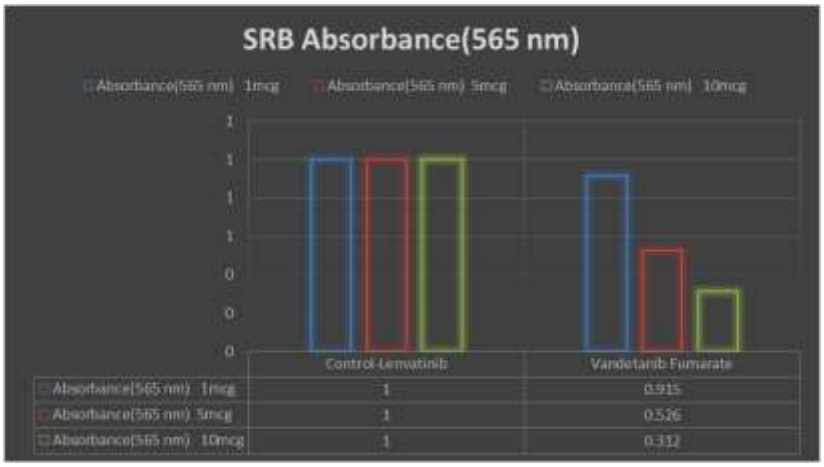
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	50,000	100%
Vandetanib Fumarate	1	0.736	37,000	74.0%
	5	0.547	27,200	54.4%
	10	0.370	16,600	33.2%

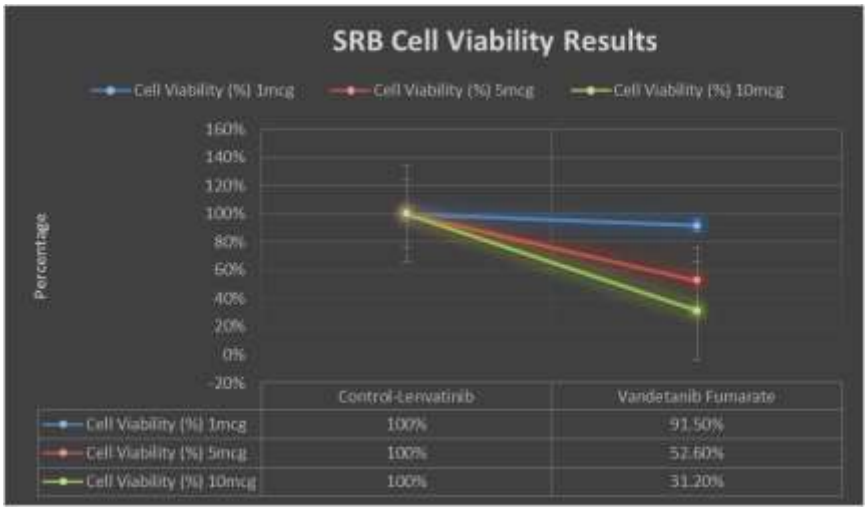




SRB Assay Results

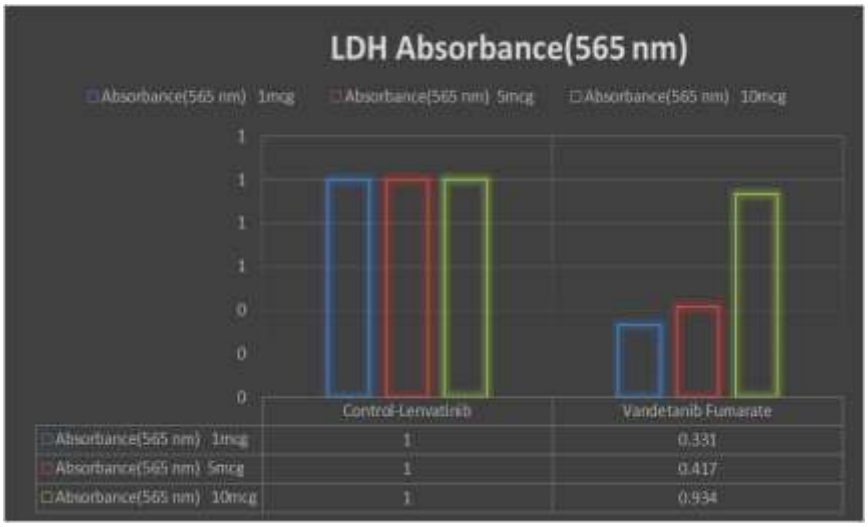
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Vandetanib Fumarate	1	0.915	91.5%
	5	0.526	52.6%
	10	0.312	31.2%

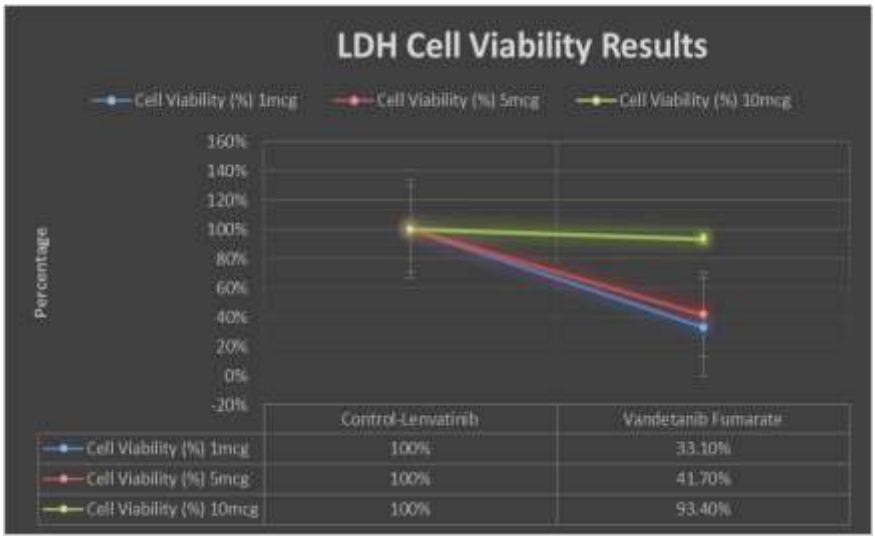




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Vandetanib Fumarate	1	0.331	33.1%
	5	0.417	41.7%
	10	0.934	93.4%





DISCUSSION

The results from this study indicate that Vandetanib Fumarate exhibits considerable cytotoxic effects and reduces cell viability in a dose-dependent manner when compared to Lenvatinib. The five assays utilized—MTT, CellTiter-Glo Luminescent, Alamar Blue, SRB, and LDH Cytotoxicity—provided comprehensive insights into Vandetanib Fumarate's impact on cell viability. Vandetanib Fumarate displayed a consistent pattern of efficacy, with higher concentrations (10 µM) showing a substantial decrease in cell viability across all assays.

The MTT assay revealed that cell viability decreased from 76.0% at 1 µM to 33.5% at 10 µM, indicating significant cytotoxicity at elevated concentrations. Similarly, in the CellTiter-Glo Luminescent assay, the cell viability decreased from 75.6% to 31.8%. The Alamar Blue assay confirmed these findings, showing a reduction in fluorescence intensity with increased Vandetanib Fumarate concentration.

The SRB and LDH assays further substantiated these observations, with the SRB assay indicating a marked reduction in cell density and the LDH assay showing increased cytotoxicity and cell death. These results collectively suggest that Vandetanib Fumarate has a comparable, if not superior, efficacy profile to Lenvatinib, particularly at higher concentrations.

CONCLUSION

The study successfully demonstrated the cytotoxic potential of Vandetanib Fumarate against kidney cancer cell lines using various in vitro assays. Vandetanib Fumarate exhibited a significant decrease in cell viability in a dose-dependent manner, indicating its promise as an alternative therapeutic agent for thyroid cancer treatment. Further in vivo studies and clinical trials are warranted to establish its safety and efficacy as a viable alternative to Lenvatinib. The findings from this study provide a strong foundation for future research and development of Vandetanib Fumarate as a targeted treatment option in thyroid cancer therapy.

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