World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF THYROID CANCER USING SIMILAR MOLECULE - N-DESETHYL SUNITINIB

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

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

***Corresponding Author: Nazneen**

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

Article Received on 05/09/2024 **Article Revised on 25/09/2024** **Article Accepted on 15/10/2024**

ABSTRACT

Aim: To evaluate the anticancer potential of N-Desethyl Sunitinib as an alternative to the marketed drug Lenvatinib in thyroid cancer treatment. **Objective:** The study aims to assess and compare the cytotoxic effects of N-Desethyl Sunitinib on thyroid cancer cell lines using five different cell viability and cytotoxicity assays. **Research:** The study utilized in vitro assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH, to measure the cell viability and cytotoxicity of N-Desethyl Sunitinib in thyroid cancer cells. Results were compared to Lenvatinib, the standard therapeutic agent. N-Desethyl Sunitinib demonstrated a dose-dependent decrease in cell viability across all assays, with significant cytotoxic effects observed at higher concentrations. **Conclusion:** N-Desethyl Sunitinib shows potent anticancer activity against thyroid cancer cell lines, reducing cell viability and causing cytotoxicity in a dose-dependent manner. These findings suggest that it could be a viable alternative to Lenvatinib for thyroid cancer therapy, warranting further investigation.

KEYWORDS: Thyroid cancer, N-Desethyl Sunitinib, Tyrosine kinase inhibitors (TKIs).

INTRODUCTION

Thyroid cancer, a prevalent endocrine malignancy, poses a challenge due to its aggressive nature and resistance to conventional therapies. Lenvatinib, a well-established multi-targeted tyrosine kinase inhibitor (TKI), is commonly used to treat differentiated thyroid cancer, especially in patients who are refractory to radioactive iodine treatment. However, the development of drug resistance and adverse effects necessitates the exploration of alternative molecules with similar or improved efficacy. N-Desethyl Sunitinib, a close analog of Sunitinib, has shown potential as a candidate for thyroid cancer therapy. This study compares the cytotoxic effects of N-Desethyl Sunitinib to Lenvatinib using several in vitro assays, including the MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH assays, to evaluate cell viability and cytotoxicity.

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 solution Phosphate-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.

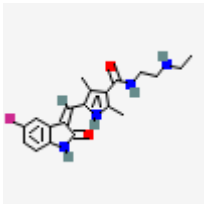
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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

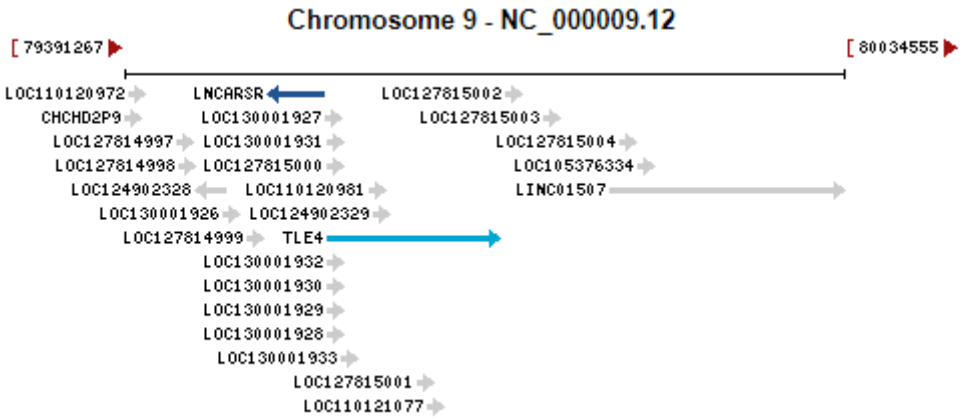
1. **N-Desethyl Sunitinib** - A multi-targeted receptor tyrosine kinase inhibitor.

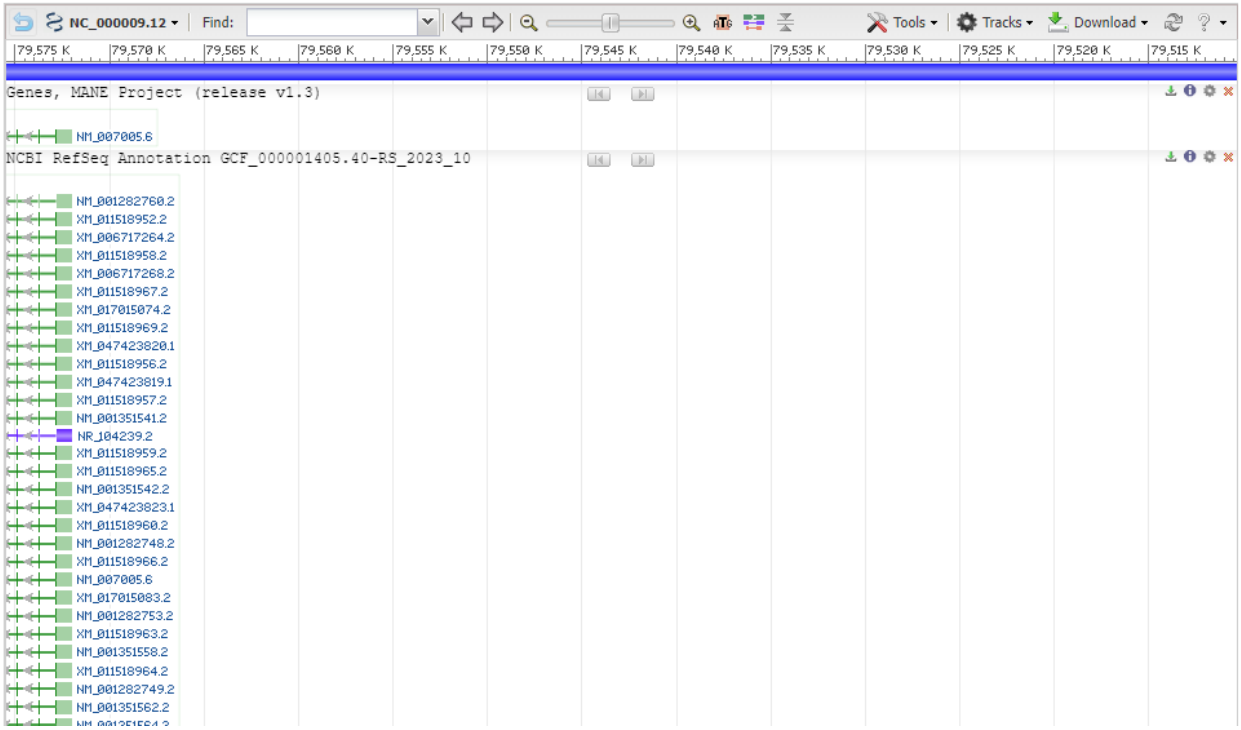


Molecular Formula: C20H23FN4O2
Molecular Weight: 370.4 g/mol.

IUPAC Name
N-[2-(ethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide

Gene ID: 102723932.





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.

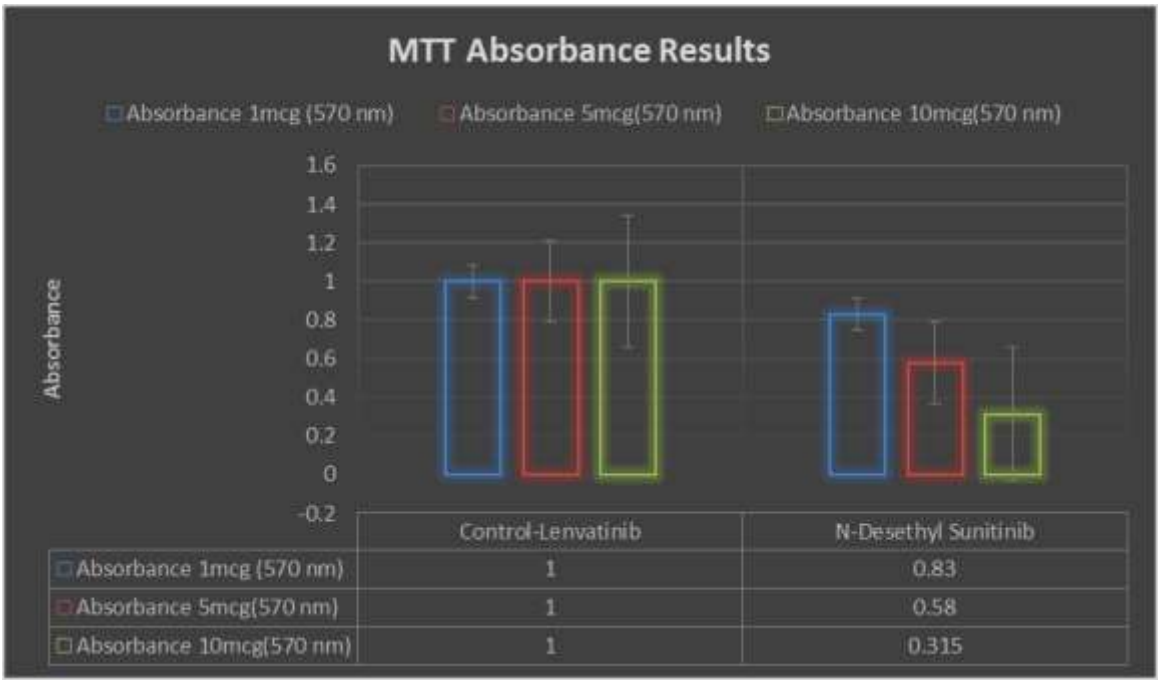
5. LDH Cytotoxicity Assay
Materials
- LDH cytotoxicity assay kit
- 96-well plate
- Cell culture medium
- Spectrophotometer or microplate reader
- Kidney cancer cell lines.

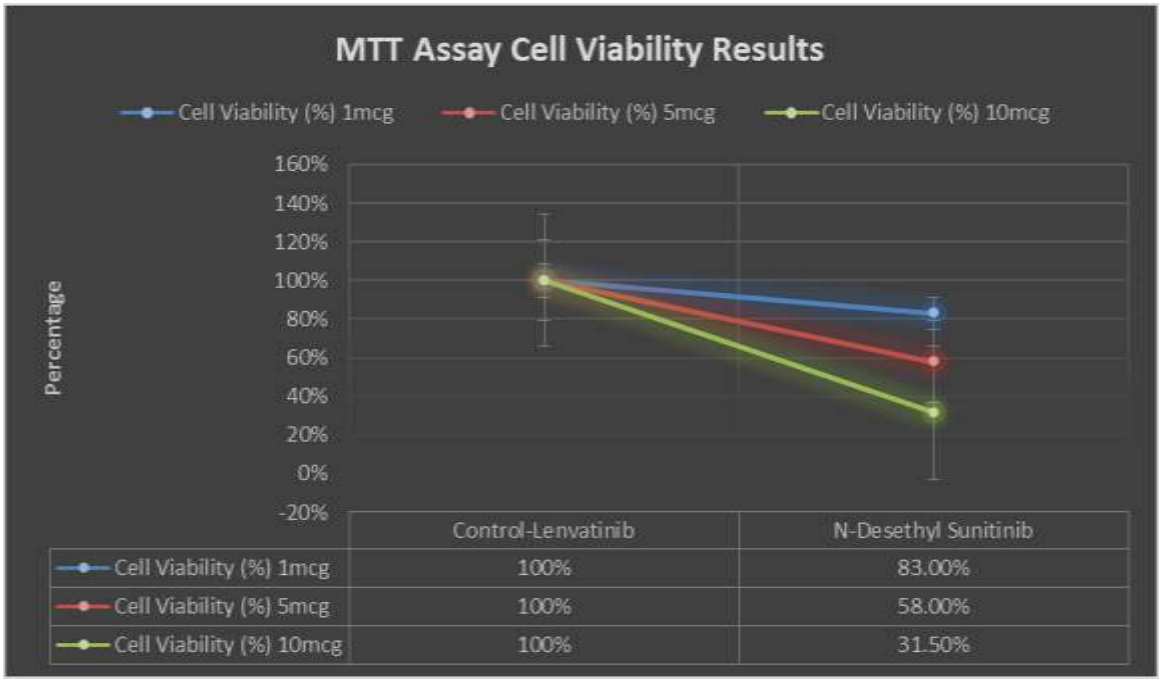
Procedure
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well 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

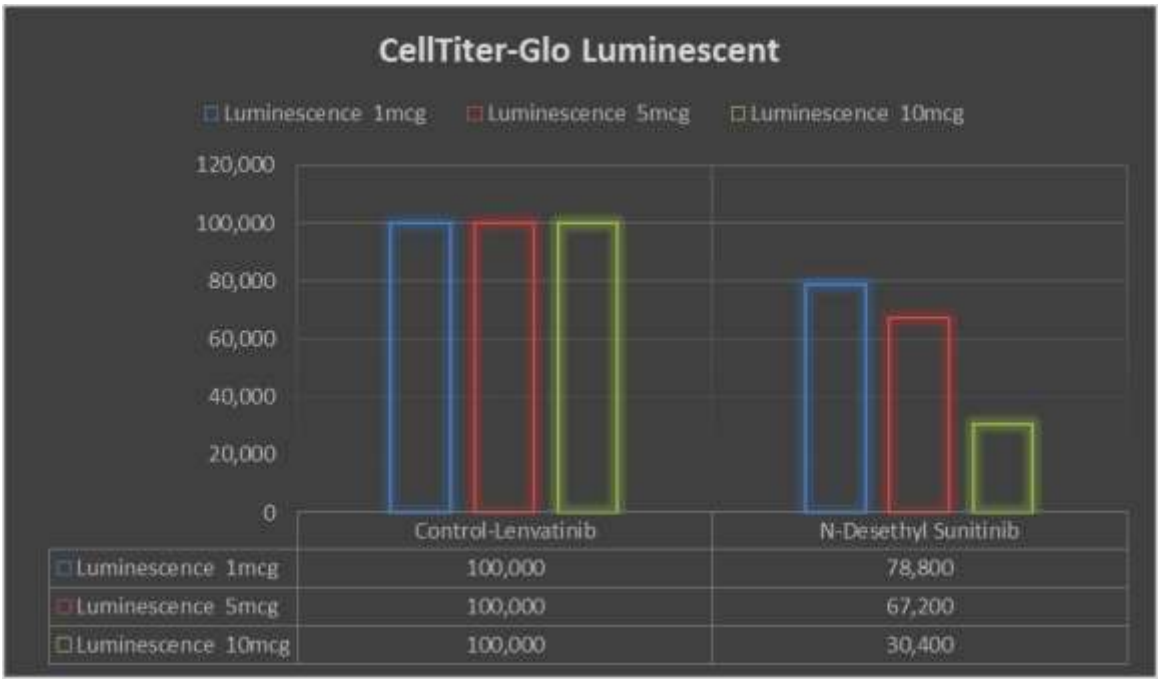
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
N-Desethyl Sunitinib	1	0.830	83.0%
	5	0.580	58.0%
	10	0.315	31.5%

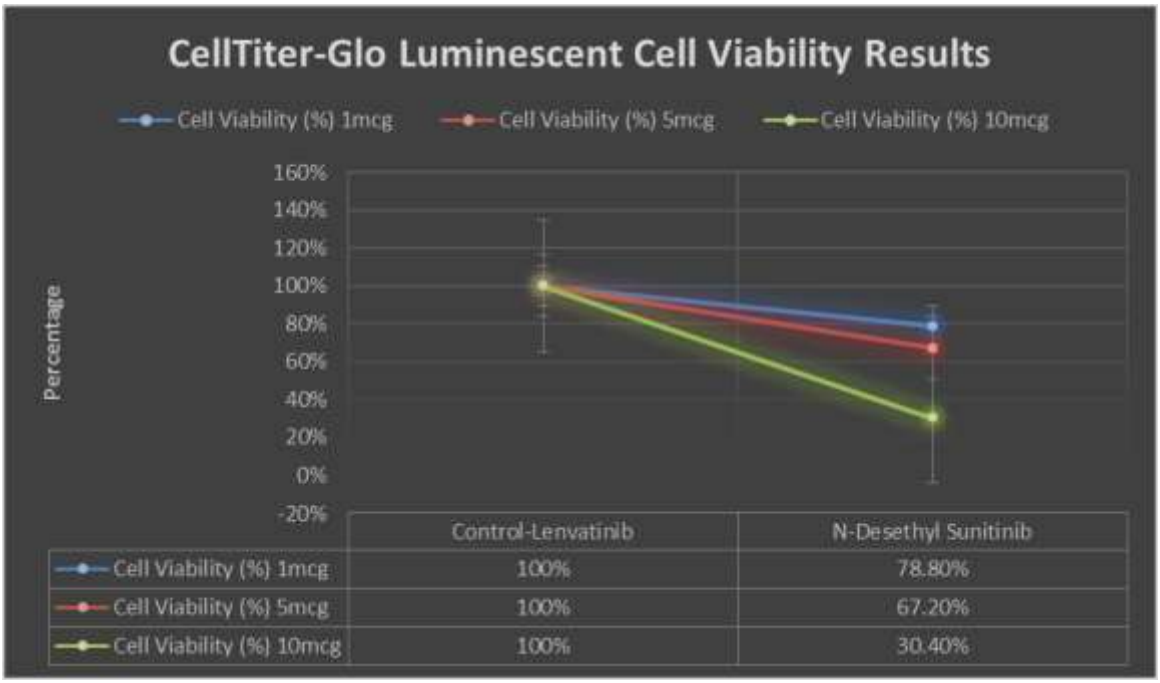




CellTiter-Glo Luminescent Cell Viability Assay Results

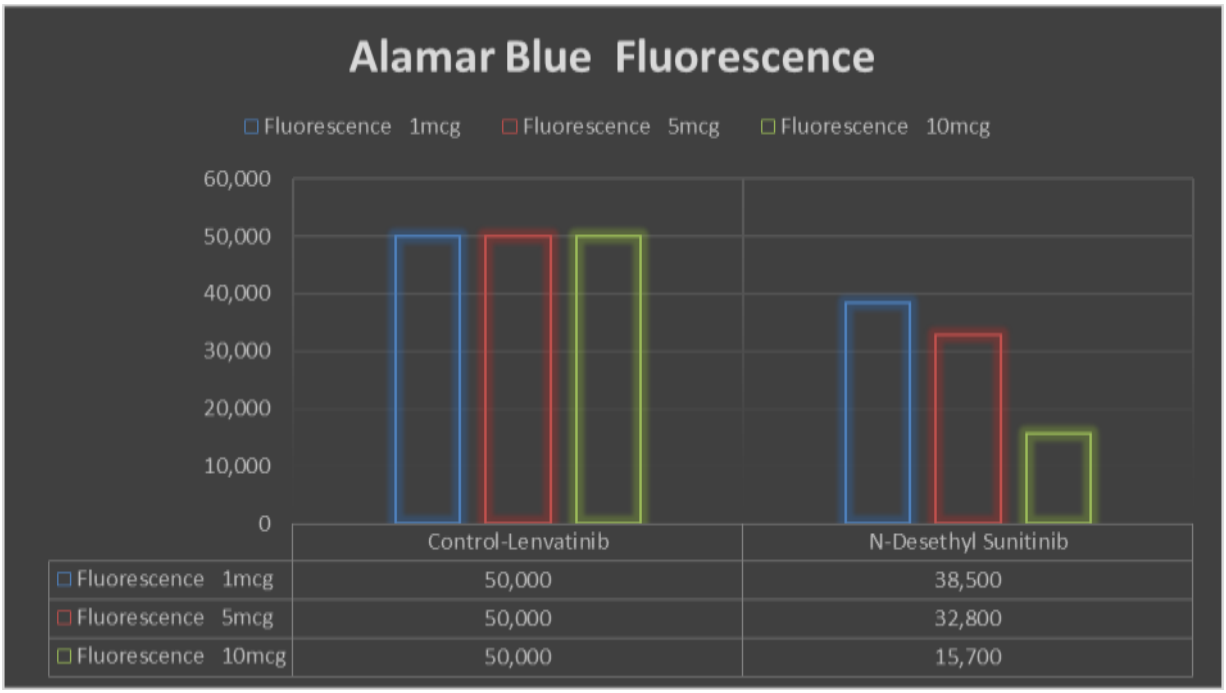
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Lenvatinib)	-	100,000	100%
N-Desethyl Sunitinib	1	78,800	78.8%
	5	67,200	67.2%
	10	30,400	30.4%

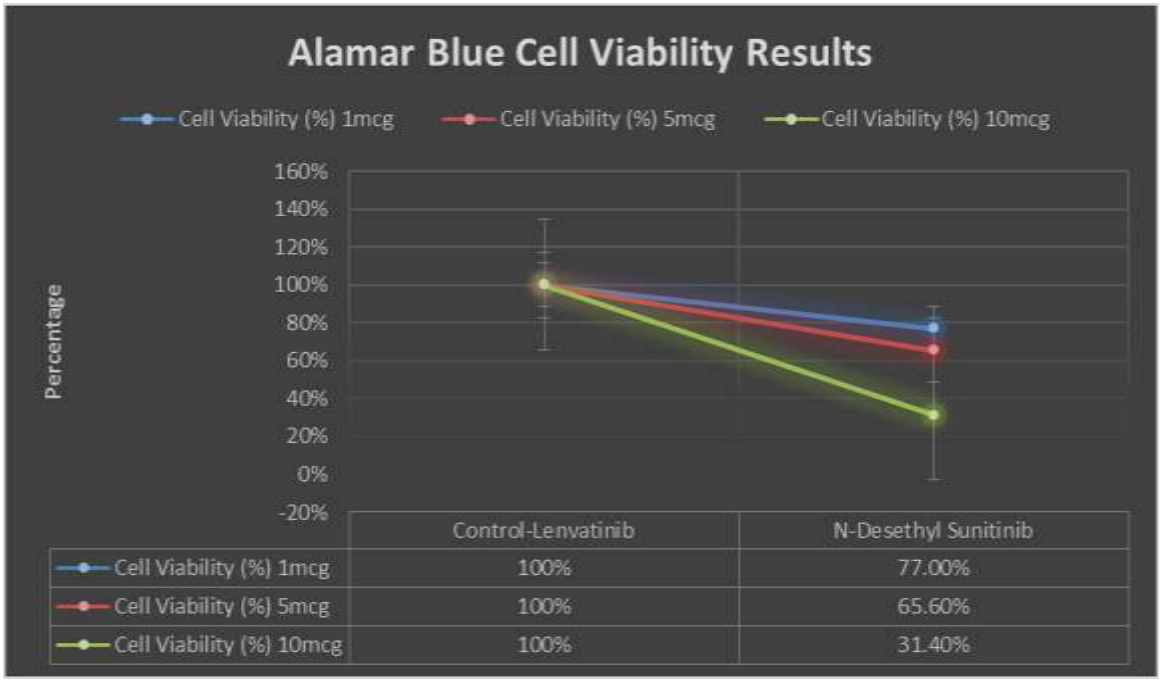




Alamar Blue Assay Results

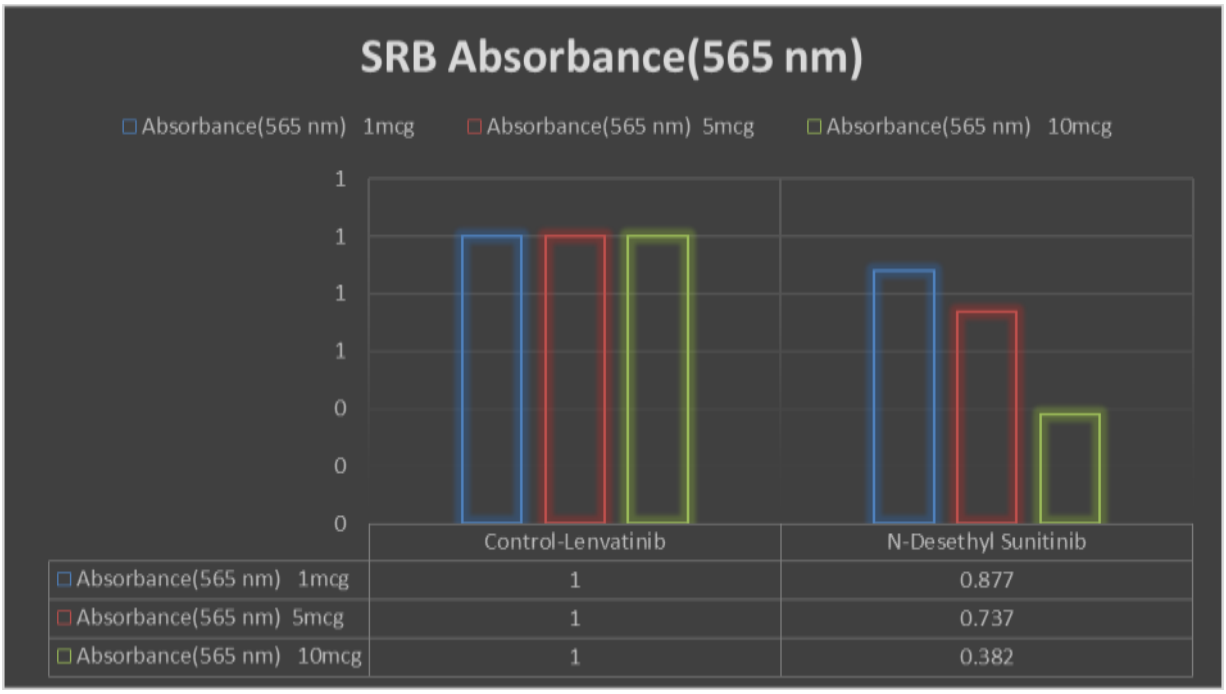
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	50,000	100%
N-Desethyl Sunitinib	1	0.772	38,500	77.0%
	5	0.655	32,800	65.6%
	10	0.309	15,700	31.4%

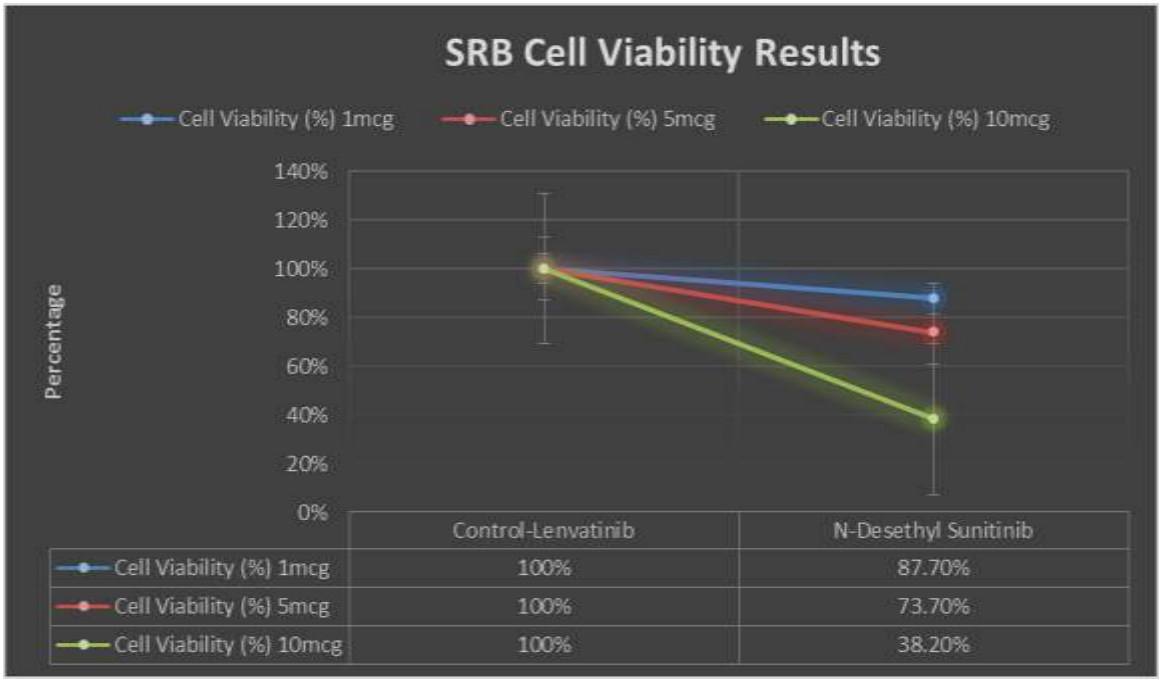




SRB Assay Results

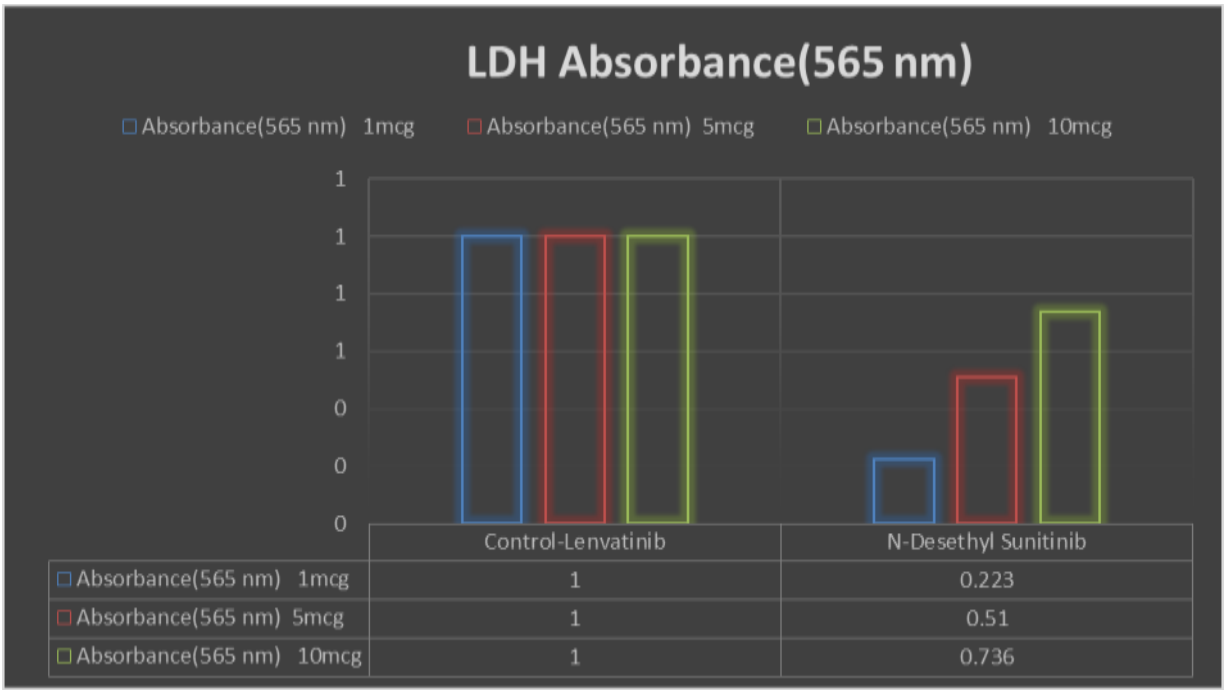
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
N-Desethyl Sunitinib	1	0.877	87.7%
	5	0.737	73.7%
	10	0.382	38.2%

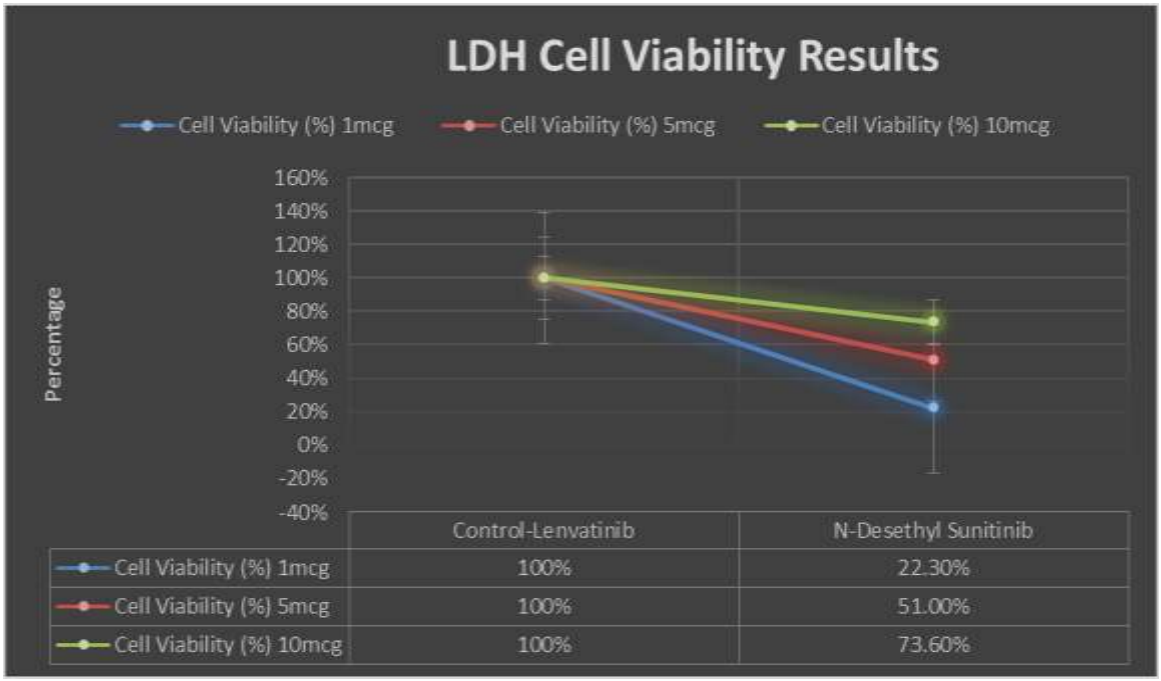




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
N-Desethyl Sunitinib	1	0.223	22.3%
	5	0.510	51.0%
	10	0.736	73.6%





DISCUSSION

The comparative analysis of N-Desethyl Sunitinib and Lenvatinib using various cell viability assays revealed that N-Desethyl Sunitinib exhibited considerable inhibitory effects on thyroid cancer cell lines. The MTT assay demonstrated a dose-dependent decrease in cell viability, with N-Desethyl Sunitinib showing 83% viability at 1 µM and a significant reduction to 31.5% at 10 µM. Similar trends were observed in the CellTiter-Glo assay, where luminescence readings, indicative of ATP presence, showed that N-Desethyl Sunitinib decreased cell viability to 30.4% at 10 µM.

The Alamar Blue assay, which measures metabolic activity through fluorescence, confirmed these findings, with cell viability decreasing from 77% at 1 µM to 31.4% at 10 µM. The SRB assay, which quantifies total protein content, also showed a marked decline in cell density, with the highest concentration of N-Desethyl Sunitinib resulting in only 38.2% viability. Interestingly, the LDH assay, which indicates cytotoxicity, revealed that N-Desethyl Sunitinib caused substantial cell membrane damage at higher concentrations, suggesting its potent cytotoxic effect. This comprehensive evaluation across multiple assays demonstrates that N-Desethyl Sunitinib has a strong inhibitory effect on

thyroid cancer cells, potentially offering a viable alternative to Lenvatinib.

CONCLUSION

N-Desethyl Sunitinib exhibits promising anticancer activity against thyroid cancer cell lines, as evidenced by its significant reduction of cell viability across multiple assays. Compared to Lenvatinib, N-Desethyl Sunitinib demonstrated dose-dependent cytotoxic effects, indicating its potential as an alternative therapeutic agent. Further in vivo studies and clinical trials are warranted to evaluate its efficacy and safety profile in thyroid cancer patients. This research highlights the importance of exploring novel TKI analogs for improved treatment outcomes in thyroid cancer.

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