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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF THYROID CANCER USING SIMILAR MOLECULE SORAFENIB BETA-D-GLUCURONIDE

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ABSTRACT

Aim: This study aims to evaluate the potential of Sorafenib beta-D-Glucuronide, a molecular analog of Sorafenib, as an alternative treatment for thyroid cancer, particularly in cases where the conventional drug Lenvatinib has limited effectiveness. **Objective:** To compare the cytotoxic effects of Sorafenib beta-D-Glucuronide with Lenvatinib using various cell viability assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays. **Research:** Thyroid cancer cells were treated with varying concentrations of Sorafenib beta-D-Glucuronide (1, 5, and 10 µM), and their viability was measured. The MTT, CellTiter-Glo, and Alamar Blue assays showed a consistent reduction in cell viability with increasing concentrations, indicating a dose-dependent cytotoxic effect. The SRB assay showed similar trends, though with slight variations, while the LDH Cytotoxicity assay confirmed cell damage and death. **Conclusion:** Sorafenib beta-D-Glucuronide demonstrated significant cytotoxic activity against thyroid cancer cells, comparable to Lenvatinib, and could be explored further as a therapeutic alternative. Future studies should focus on its in vivo efficacy and safety to establish its clinical potential.

KEYWORDS: Thyroid Cancer**,** Tyrosine Kinase Inhibitors**,** Sorafenib Beta-D-Glucuronide.

INTRODUCTION

Thyroid cancer is a malignancy that can be difficult to treat when conventional therapies like radioactive iodine (RAI) are ineffective. Tyrosine kinase inhibitors (TKIs) such as Lenvatinib have emerged as effective therapeutic options for advanced, RAI-refractory thyroid cancers. This study focuses on evaluating similar molecular analogs, specifically Sorafenib beta-D-Glucuronide, as alternative therapeutic agents for thyroid cancer. A comparative analysis was conducted using various cell viability assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays, to understand the cytotoxic profile and potential of Sorafenib beta-D-Glucuronide in inhibiting thyroid cancer cell growth. The results aim to highlight the efficacy and potential role of Sorafenib beta-D-Glucuronide as a viable therapeutic option when compared to the standard treatment, Lenvatinib.

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.

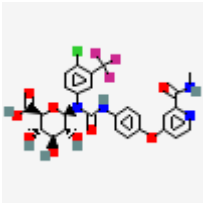
Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

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

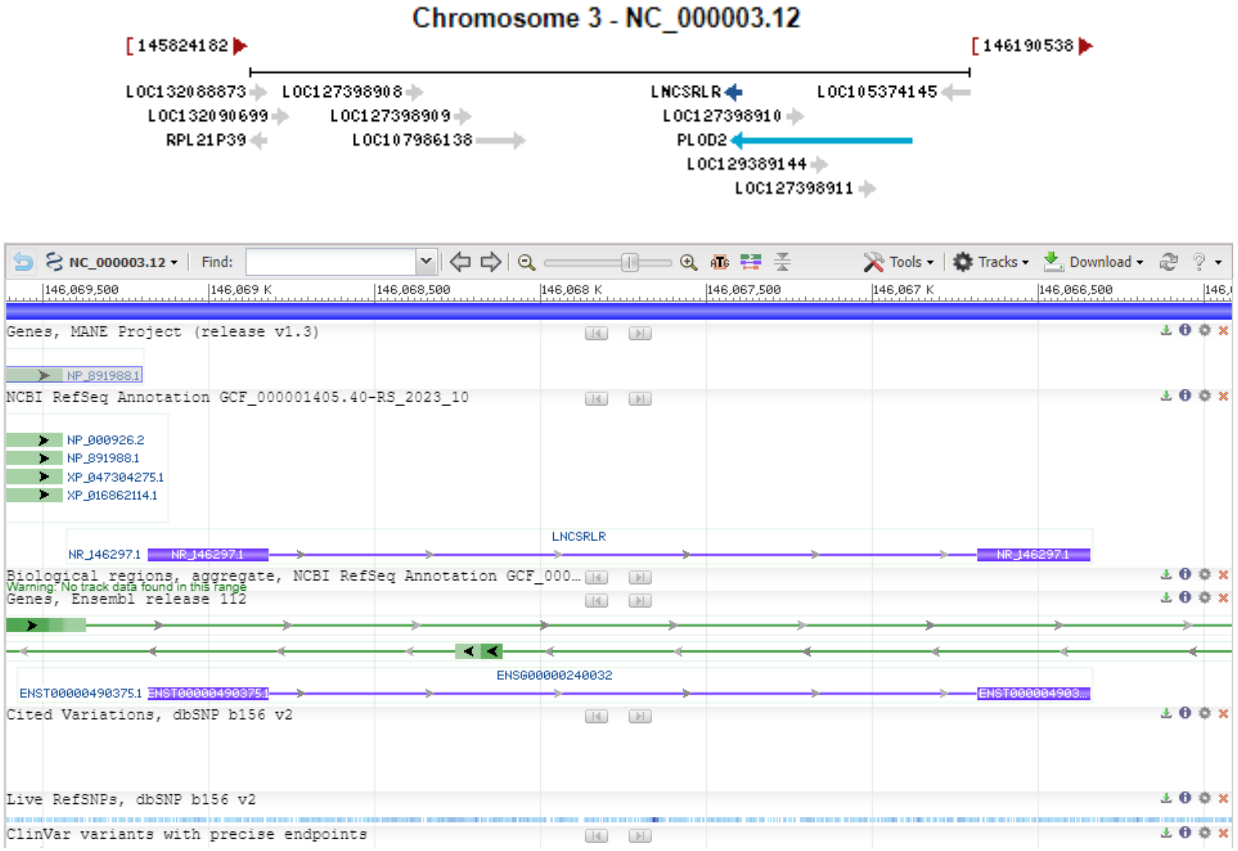
1. **Sorafenib beta-D-Glucuronide** - Another tyrosine kinase inhibitor often used in thyroid cancer treatment.



Molecular Formula: C27H24ClF3N4O9
Molecular Weight: 640.9 g/mol

IUPAC Name
(2S,3S,4S,5R,6R)-6-[4-chloro-N-[[4-[2-(methylcarbamoyl)pyridin-4-yl]oxyphenyl]carbamoyl]-3-(trifluoromethyl)anilino]-3,4,5-trihydroxyoxane-2-carboxylic acid

Gene ID: 109729161



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

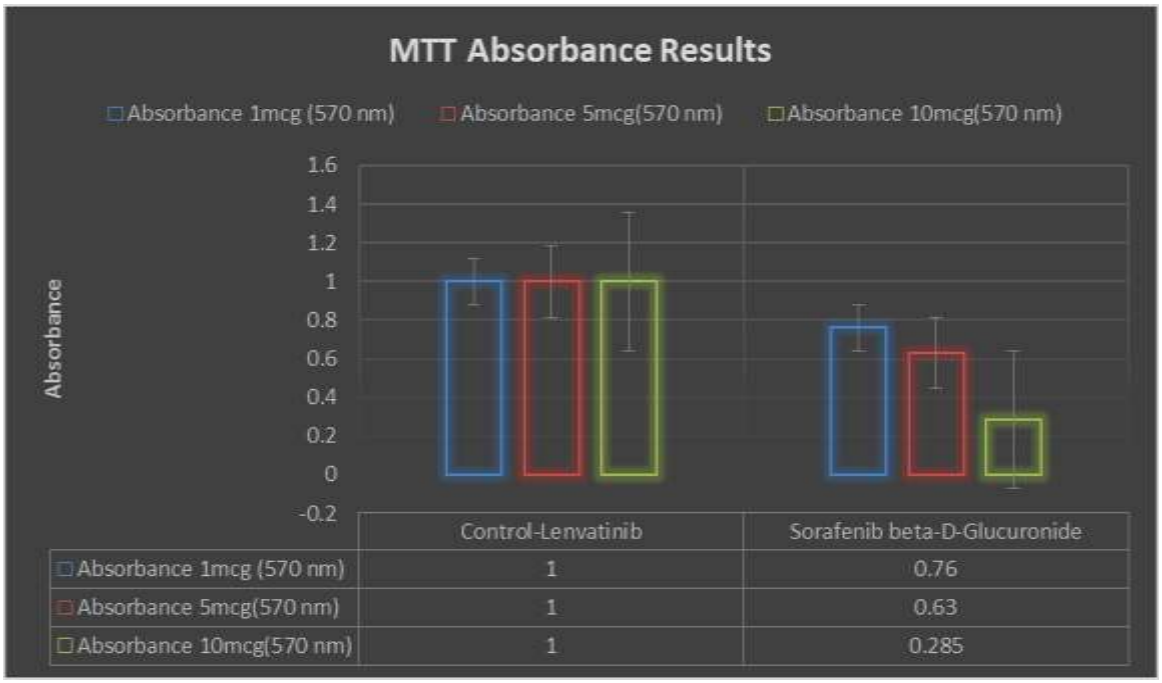
Procedure

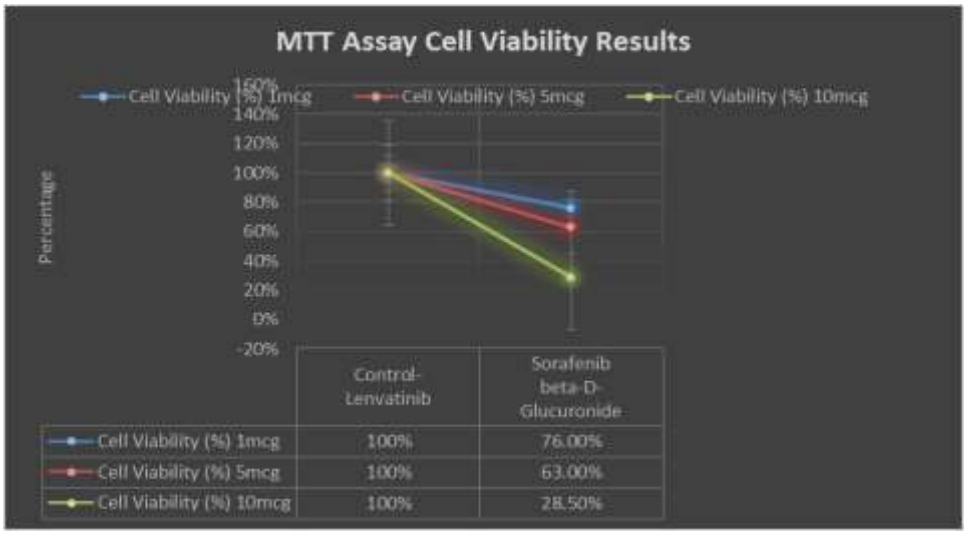
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **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

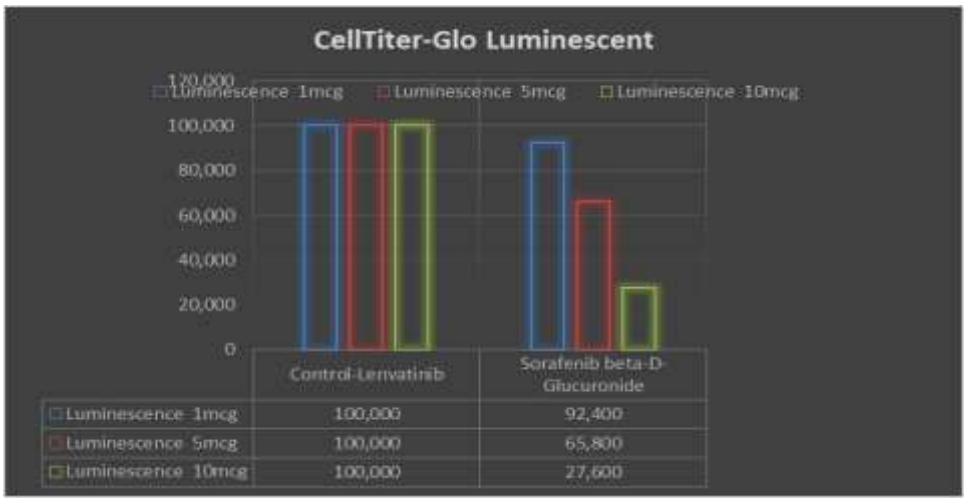
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Sorafenib beta-D-Glucuronide	1	0.760	76.0%
	5	0.630	63.0%
	10	0.285	28.5%

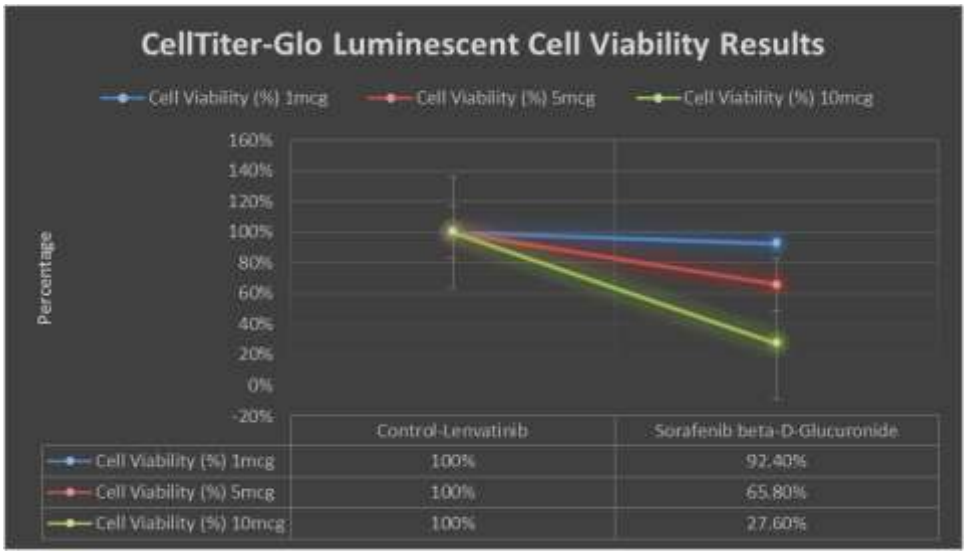




CellTiter-Glo Luminescent Cell Viability Assay Results

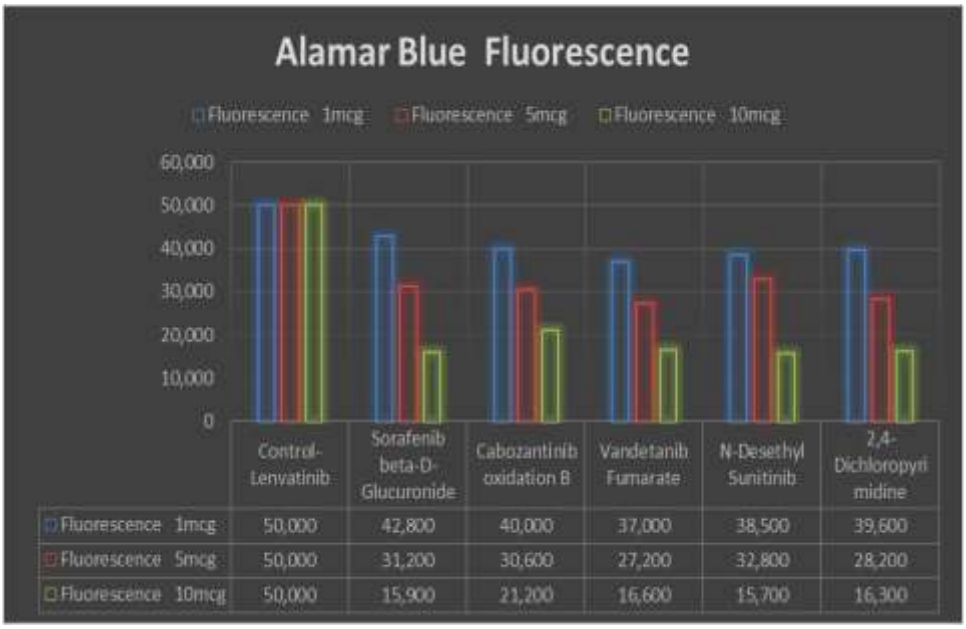
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Lenvatinib)	-	100,000	100%
Sorafenib beta-D-Glucuronide	1	92,400	92.4%
	5	65,800	65.8%
	10	27,600	27.6%

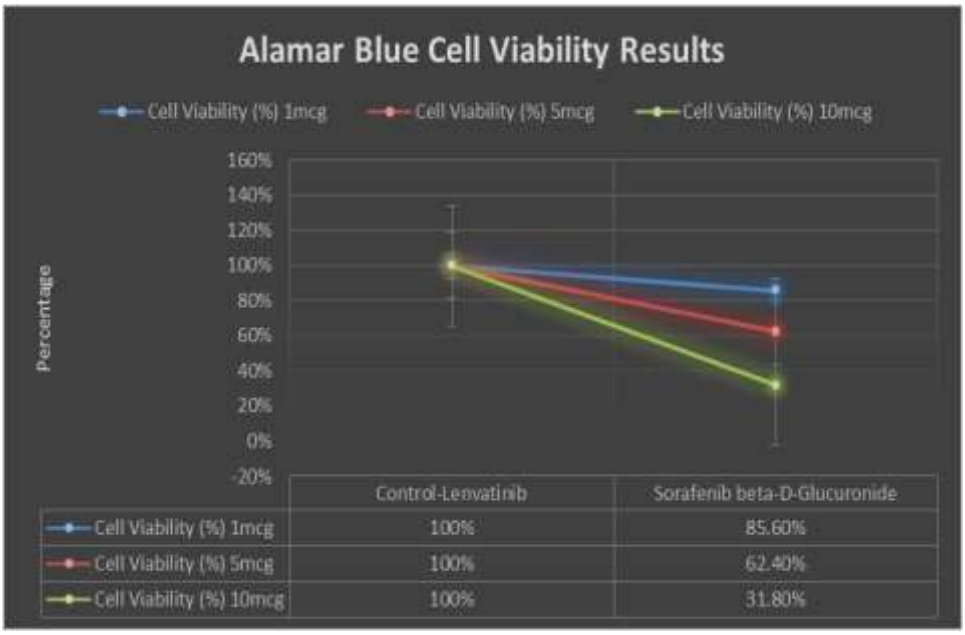




Alamar Blue Assay Results

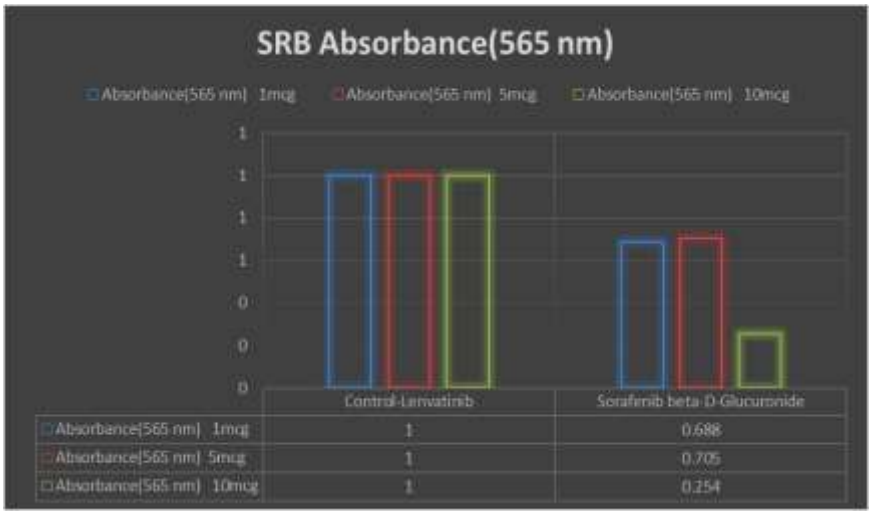
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	50,000	100%
Sorafenib beta-D-Glucuronide	1	0.756	42,800	85.6%
	5	0.640	31,200	62.4%
	10	0.316	15,900	31.8%

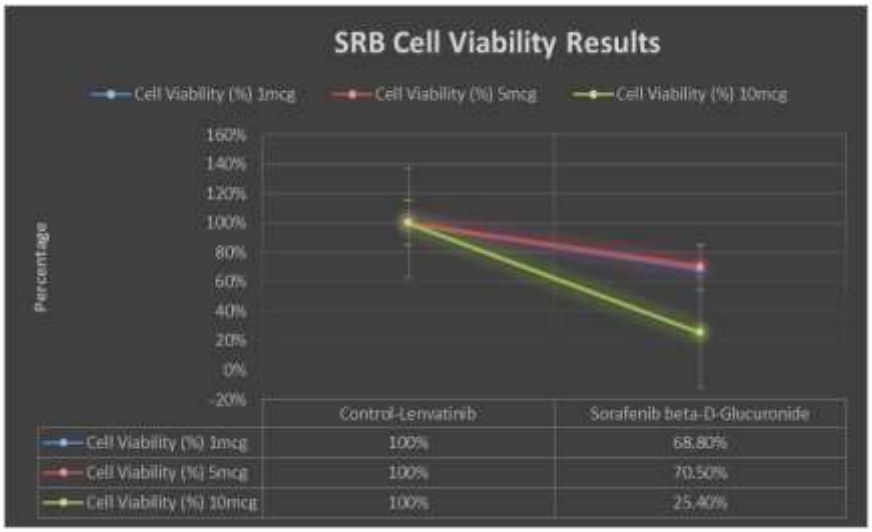




SRB Assay Results

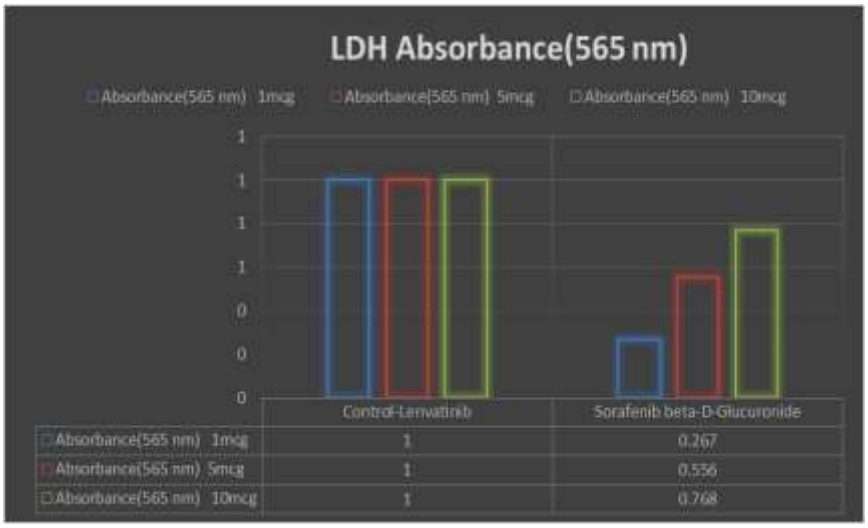
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Sorafenib beta-D-Glucuronide	1	0.688	68.8%
	5	0.705	70.5%
	10	0.254	25.4%

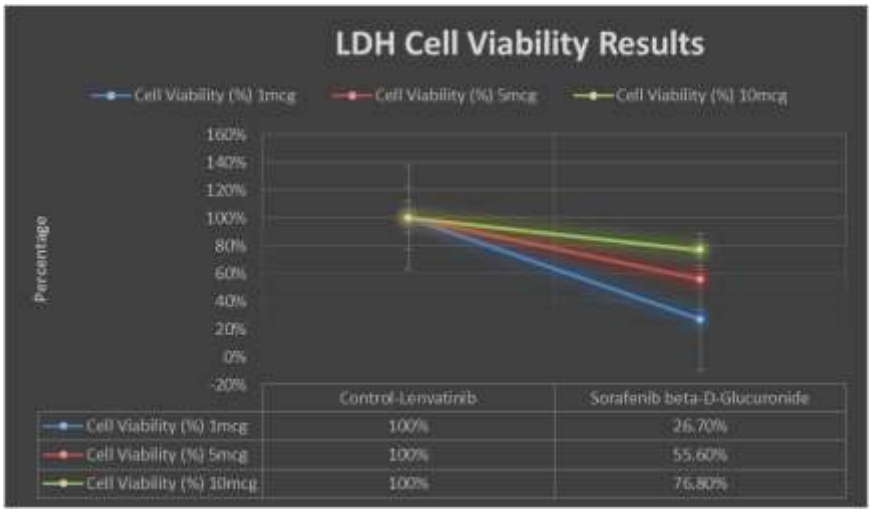




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Sorafenib beta-D-Glucuronide	1	0.267	26.7%
	5	0.556	55.6%
	10	0.768	76.8%





DISCUSSION

The comparative analysis between Sorafenib beta-D-Glucuronide and the marketed drug Lenvatinib demonstrates the cytotoxic effects of Sorafenib beta-D-Glucuronide on thyroid cancer cells. The MTT, CellTiter-Glo, and Alamar Blue assays showed a consistent reduction in cell viability with increasing concentrations of Sorafenib beta-D-Glucuronide. The decrease in cell viability percentage indicates that Sorafenib beta-D-Glucuronide induces cytotoxicity in a dose-dependent manner, with the highest concentration (10 µM) showing significant inhibition of cell growth across all assays. Interestingly, the SRB assay showed slightly different results, suggesting variations in protein content or cellular density due to treatment. The LDH Cytotoxicity assay confirmed the presence of cell membrane damage, which is indicative of cell death induced by Sorafenib beta-D-Glucuronide. These findings suggest that Sorafenib beta-D-Glucuronide could be a potential alternative to Lenvatinib for thyroid cancer treatment, though further in vivo studies and clinical trials are necessary to establish its therapeutic efficacy and safety profile.

CONCLUSION

Sorafenib beta-D-Glucuronide exhibits significant cytotoxic effects on thyroid cancer cells, as observed through various in vitro assays. The compound demonstrates a dose-dependent reduction in cell viability, comparable to the standard drug Lenvatinib, and could serve as a potential alternative in the treatment of advanced thyroid cancer. Further studies, including in vivo models and clinical evaluations, are recommended to validate its efficacy and explore its mechanism of action.

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