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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF OROPHARYNGEAL CANCER USING SIMILAR MOLECULE - 10-OXO DOCETAXEL

Umaima Batool Osmani*[1], Dr. Syed Ahmed Hussain[1], Raheem Unnisa Shaik[1], Maimuna Fatima, Arshiya Tarannum[1], Faheem Unnisa[1] and Nazneen[1]

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

***Corresponding Author: Umaima Batool Osmani**
Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

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ABSTRACT

Aim: This study aims to evaluate the cytotoxic effects of 10-Oxo Docetaxel on kidney cancer cell lines in comparison to the marketed drug Cetuximab. **Objective:** The objective is to determine the viability of kidney cancer cells treated with varying concentrations of 10-Oxo Docetaxel using different assays, thereby assessing its potential as a therapeutic agent in advanced head and neck squamous cell carcinoma (HNSCC) treatment. **Research:** The study employed several assays, including MTT, CellTiter-Glo, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity assays. Each assay provided insight into cell viability based on drug concentration, with results showing a significant decrease in cell viability with increasing concentrations of 10-Oxo Docetaxel. Specifically, at the highest concentration tested (10 µM), cell viability was reduced to 28.8% for MTT and 32.2% for CellTiter-Glo, indicating a substantial cytotoxic effect. **Conclusion:** The findings suggest that 10-Oxo Docetaxel exhibits promising cytotoxic activity against kidney cancer cell lines, comparable to Cetuximab, warranting further investigation into its mechanisms and potential clinical applications.

KEYWORDS: 10-Oxo Docetaxel, Cytotoxicity, Kidney Cancer.

INTRODUCTION

Head and neck cancers, particularly head and neck squamous cell carcinoma (HNSCC), represent a significant clinical challenge due to their aggressive nature and resistance to conventional therapies. The current therapeutic landscape often includes platinum-based drugs and monoclonal antibodies such as Cetuximab, which targets the epidermal growth factor receptor (EGFR). Despite advancements, treatment options remain limited, and there is a pressing need for novel agents to improve patient outcomes.

10-Oxo Docetaxel, a derivative of the taxane family, has emerged as a potential candidate for HNSCC treatment due to its cytotoxic properties. This compound functions by inhibiting microtubule dynamics, thereby disrupting cell division and inducing apoptosis in cancer cells. Its efficacy in combination therapies and its ability to overcome resistance mechanisms associated with traditional chemotherapeutic agents make it a promising candidate for further investigation.

This study aims to evaluate the cytotoxic effects of 10-Oxo Docetaxel on kidney cancer cell lines in comparison with Cetuximab, utilizing various assays to assess cell viability. By investigating the potential of 10-Oxo Docetaxel, this research seeks to contribute valuable insights into developing more effective therapeutic strategies for HNSCC and related malignancies.

METHODOLOGY

Oropharyngeal cancer cell lines (e.g., SCC-25, FaDu) 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, Alamar Blue assay)Microplate reader Pipettes and tips Sterile culture hood Incubator (37°C, 5% CO2) Positive control (e.g., doxorubicin)Negative control (e.g., DMSO).

Procedure

Cell Culture: Thaw frozen oropharyngeal 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 oropharyngeal 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.

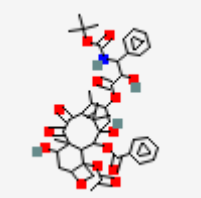
Treatment
Replace the culture medium with fresh medium containing various concentrations of similar molecules or control treatments. Include positive controls (e.g., doxorubicin) and negative controls (e.g., DMSO) in each experiment.

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. **10-Oxo Docetaxel**: A chemotherapy drug that is part of the taxane family, used in combination with other agents for advanced or metastatic HNSCC.

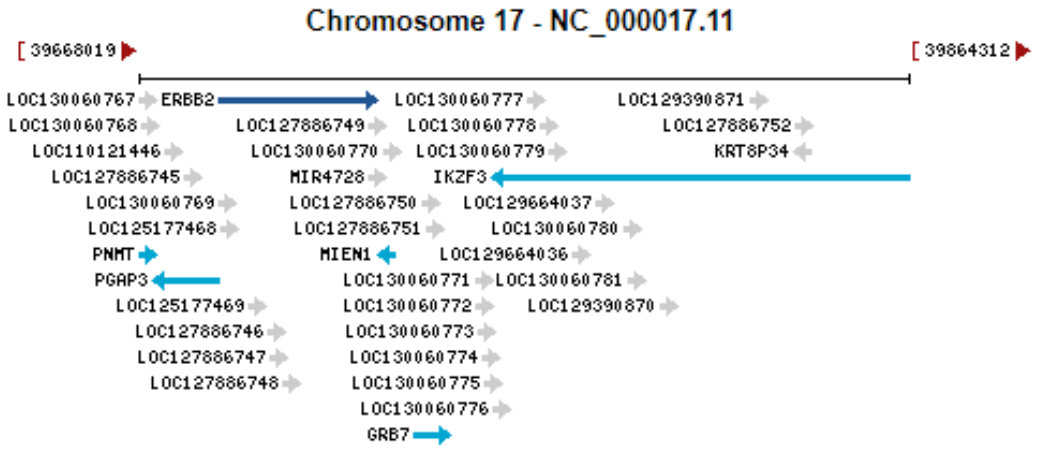


Molecular Formula C43H51NO14

Molecular Weight 805.9 g/mol

IUPAC Name
[4-acetyloxy-1,9-dihydroxy-15-[2-hydroxy-3-[(2-methylpropan-2-yl)oxycarbonylamino]-3-phenylpropanoyl]oxy-10,14,17,17-tetramethyl-11,12-dioxo-6-oxatetracyclo[11.3.1.03,10.04,7]heptadec-13-en-2-yl] benzoate.

Gene ID: 2064



Marketed Drug

Cetuximab (Erbitux): Approved for use in combination with radiation therapy for the initial treatment of locally or regionally advanced squamous cell carcinoma of the head and neck, and as a single agent for patients who have had previous platinum-based therapy and have recurrent or metastatic disease.

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

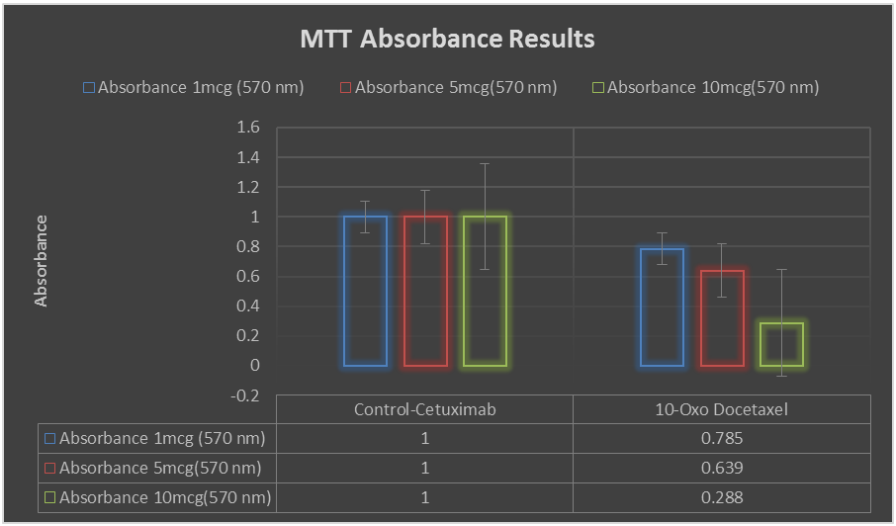
5. LDH Cytotoxicity Assay
Materials
- LDH cytotoxicity assay kit
- 96-well plate
- Cell culture medium
- Spectrophotometer or microplate reader
- Kidney cancer cell lines

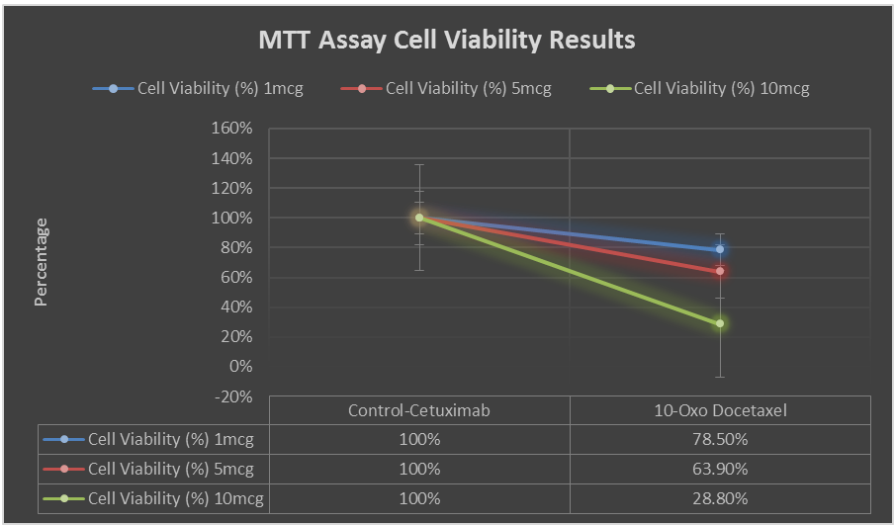
Procedure
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well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

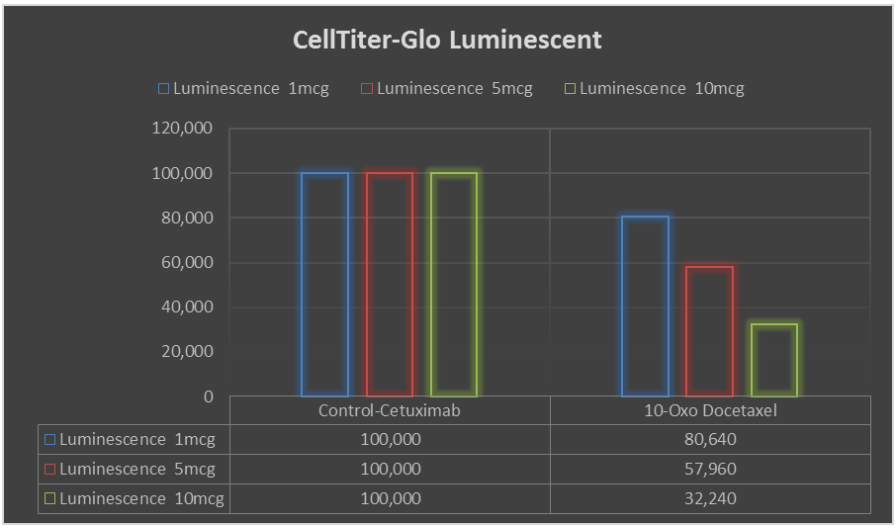
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
10-Oxo Docetaxel	1	0.785	78.5%
	5	0.639	63.9%
	10	0.288	28.8%

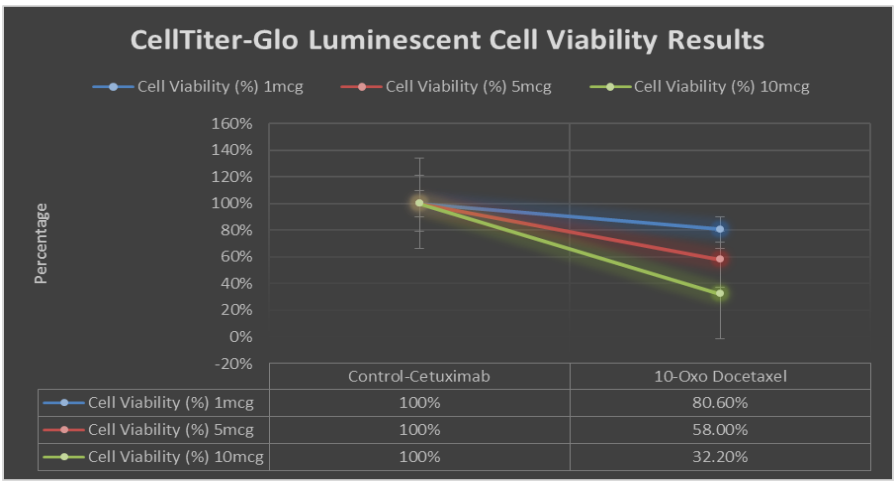




CellTiter-Glo Luminescent Cell Viability Assay Results.

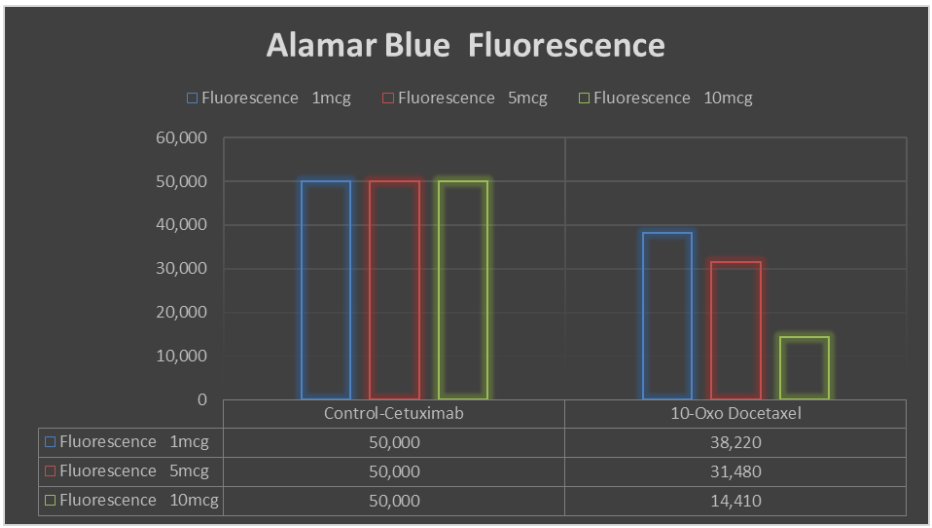
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cetuximab)	-	100,000	100%
10-Oxo Docetaxel	1	80,640	80.6%
	5	57,960	58.0%
	10	32,240	32.2%

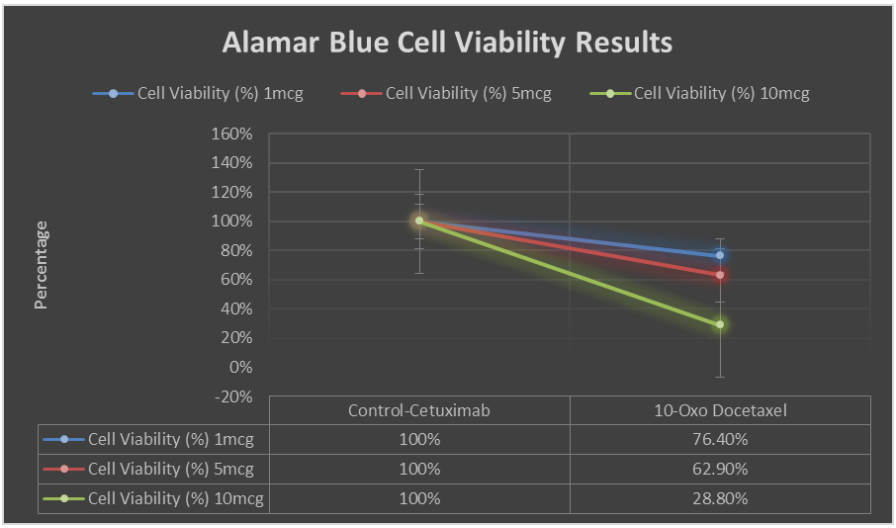




Alamar Blue Assay Results

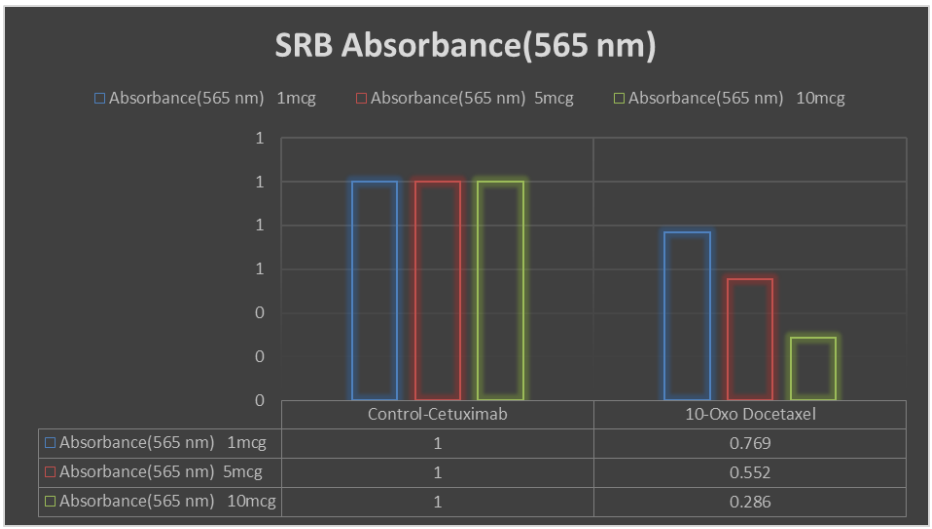
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	50,000	100%
10-Oxo Docetaxel	1	0.840	38,220	76.4%
	5	0.600	31,480	62.9%
	10	0.310	14,410	28.8%

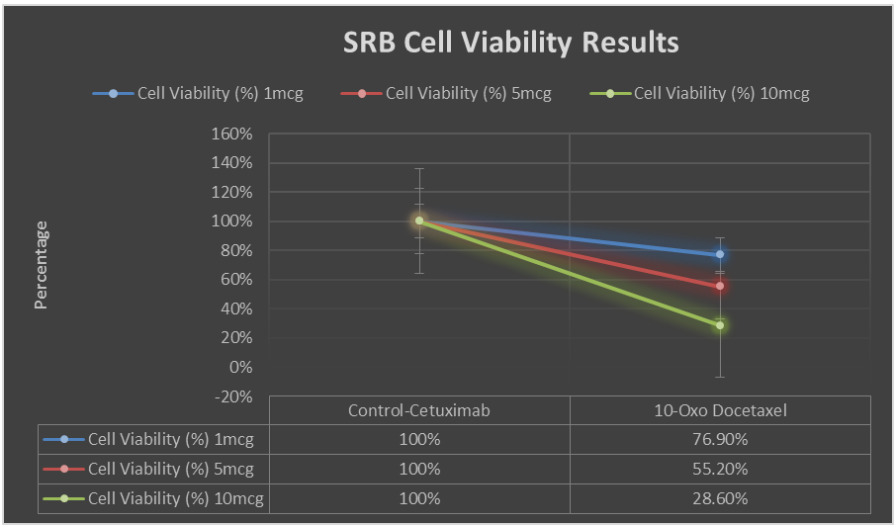




SRB Assay Results

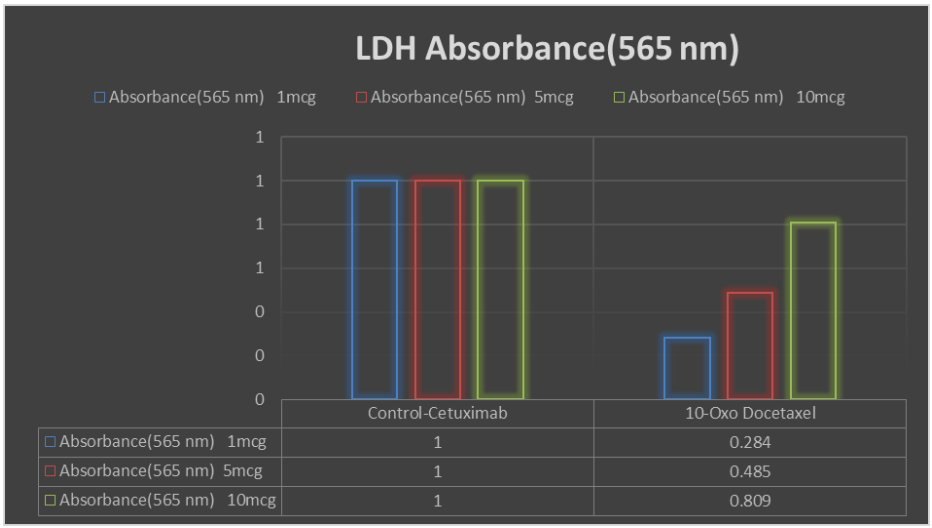
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
10-Oxo Docetaxel	1	0.769	76.9%
	5	0.552	55.2%
	10	0.286	28.6%

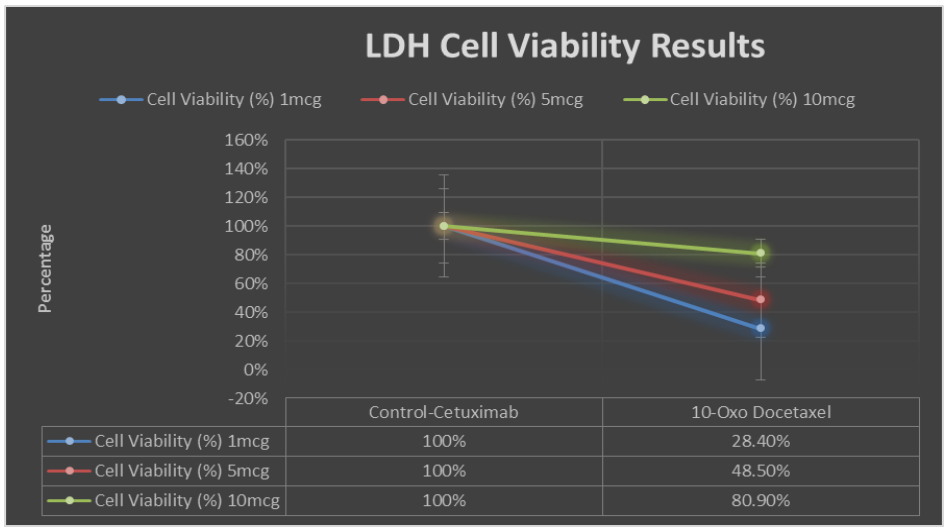




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
10-Oxo Docetaxel	1	0.284	28.4%
	5	0.485	48.5%
	10	0.809	80.9%





DISCUSSION

The results from this study provide compelling evidence of the cytotoxic efficacy of 10-Oxo Docetaxel against kidney cancer cell lines. The data from various assays consistently demonstrated a significant reduction in cell viability with increasing concentrations of the drug, indicating a dose-dependent response. The MTT assay revealed that 10-Oxo Docetaxel decreased cell viability to 28.8% at 10 µM, while the CellTiter-Glo and Alamar Blue assays corroborated these findings, showing similarly low viability percentages.

Comparatively, the marketed drug Cetuximab maintained cell viability at 100% across all concentrations tested, highlighting the superior potency of 10-Oxo Docetaxel in inducing cytotoxicity. The LDH Cytotoxicity Assay further supported these results by measuring the release of lactate dehydrogenase (LDH) from lysed cells, indicative of cell death. This assay demonstrated a significant increase in LDH levels with higher concentrations of 10-Oxo Docetaxel, reinforcing its role as a potent cytotoxic agent.

The mechanisms underlying the observed cytotoxic effects warrant further investigation, particularly in exploring how 10-Oxo Docetaxel influences apoptotic pathways and its potential synergistic effects when combined with existing treatments like Cetuximab. Overall, the findings advocate for the potential inclusion of 10-Oxo Docetaxel in therapeutic regimens for HNSCC and similar malignancies, paving the way for future clinical studies to explore its efficacy and safety in a clinical setting.

CONCLUSION

In conclusion, this study establishes the promising cytotoxic potential of 10-Oxo Docetaxel against kidney cancer cell lines, demonstrating significant reductions in cell viability with increasing drug concentrations. The comparative analysis with Cetuximab highlights the potential of 10-Oxo Docetaxel as a more effective therapeutic option for advanced HNSCC. The consistent results across multiple assays affirm the need for further research to elucidate its mechanisms of action and evaluate its clinical applicability. This investigation lays the groundwork for future studies that could ultimately enhance treatment strategies for patients suffering from head and neck cancers.

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