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

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ABSTRACT

Aim: The aim of this study is to explore and evaluate the efficacy of alternative molecules similar to lenvatinib in the treatment of thyroid cancer, focusing on *Cabozantinib oxidation B*, a potential tyrosine kinase inhibitor. **Objective**: To compare the anticancer effects of *Cabozantinib oxidation B* using various cell viability assays, providing insights into its effectiveness in inhibiting thyroid cancer cell proliferation. **Research**: Five commonly used cell viability assays, namely MTT, CellTiter-Glo Luminescent, Alamar Blue, Sulforhodamine B (SRB), and LDH Cytotoxicity assays, were employed to analyze the cytotoxic and proliferative effects of *Cabozantinib oxidation B* on cancer cells. The results were compared with those obtained using lenvatinib as the control. The assays revealed that *Cabozantinib oxidation B* showed a concentration-dependent decrease in cell viability, exhibiting significant inhibition at higher concentrations. **Conclusion**: *Cabozantinib oxidation B* demonstrated substantial cytotoxicity and cell viability inhibition across all assays, making it a promising candidate for further research in thyroid cancer treatment as an alternative to lenvatinib.

KEYWORDS

1. Thyroid cancer treatment
2. Tyrosine kinase inhibitor
3. Cabozantinib oxidation B

INTRODUCTION

Thyroid cancer is a prevalent endocrine malignancy, and its treatment often involves tyrosine kinase inhibitors, especially in cases resistant to conventional therapies like radioactive iodine. Lenvatinib, a multi-targeted tyrosine kinase inhibitor, has shown significant efficacy in treating differentiated thyroid cancer. However, resistance to lenvatinib and its associated side effects have led to the search for alternative therapeutic agents. This study investigates *Cabozantinib oxidation B*, a molecule structurally similar to lenvatinib, for its potential as a therapeutic agent in thyroid cancer. The primary objective of this study is to evaluate the efficacy of *Cabozantinib oxidation B* in inhibiting thyroid cancer cell proliferation using a series of cytotoxicity and viability 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).

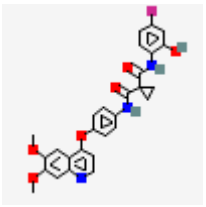
Incubate the plates for an additional period to allow the formation of formazan crystals or the reduction of resazurin.

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:

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

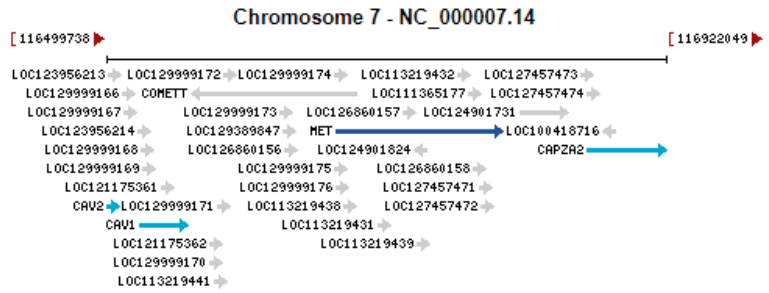
Similar Molecules
1. **Cabozantinib oxidation B** - Used for medullary thyroid cancer.



Molecular Formula: C28H24FN3O6
Molecular Weight: 517.5 g/mol

IUPAC Name
1-N-[4-(6,7-dimethoxyquinolin-4-yl)oxyphenyl]-1-N'-(4-fluoro-2-hydroxyphenyl)cyclopropane-1,1-dicarboxamide.

Gene ID: 4233



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

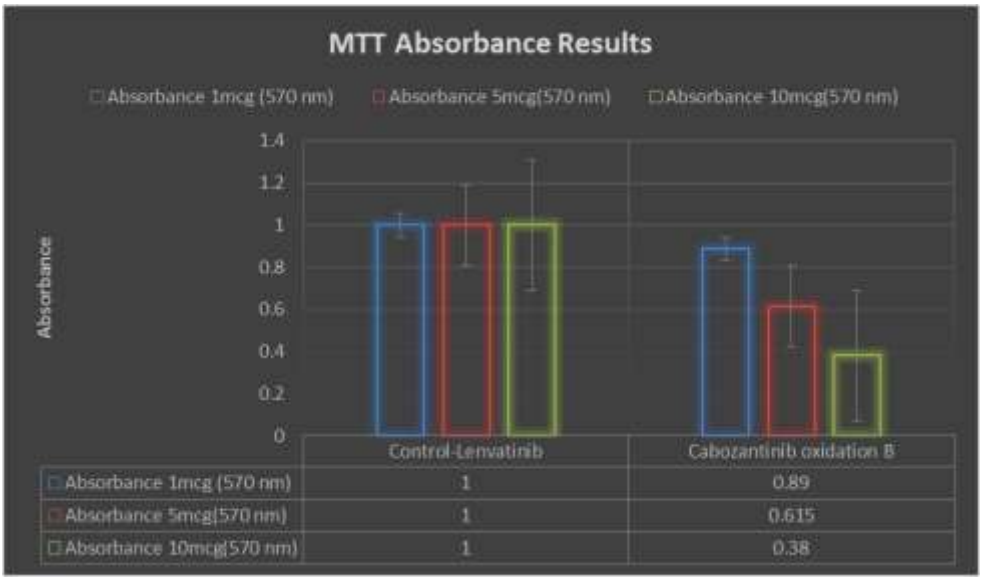
Procedure

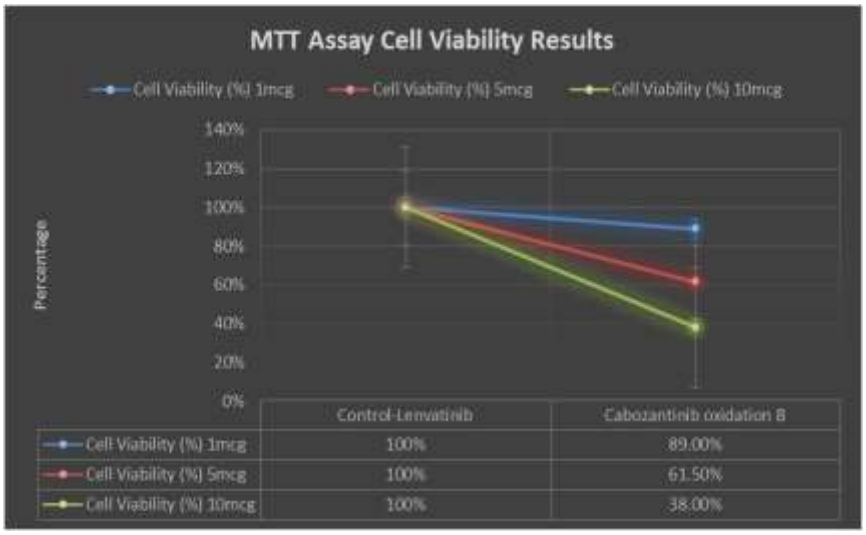
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4.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

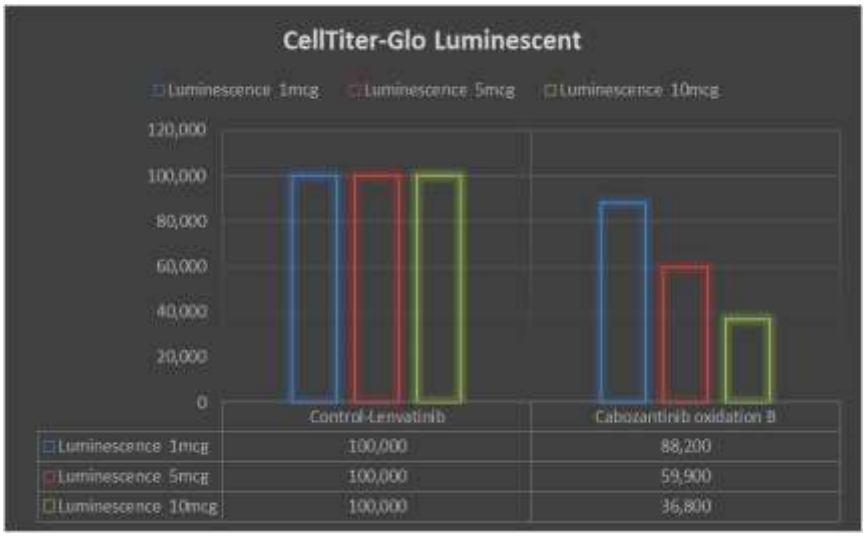
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Cabozantinib oxidation B	1	0.890	89.0%
	5	0.615	61.5%
	10	0.380	38.0%

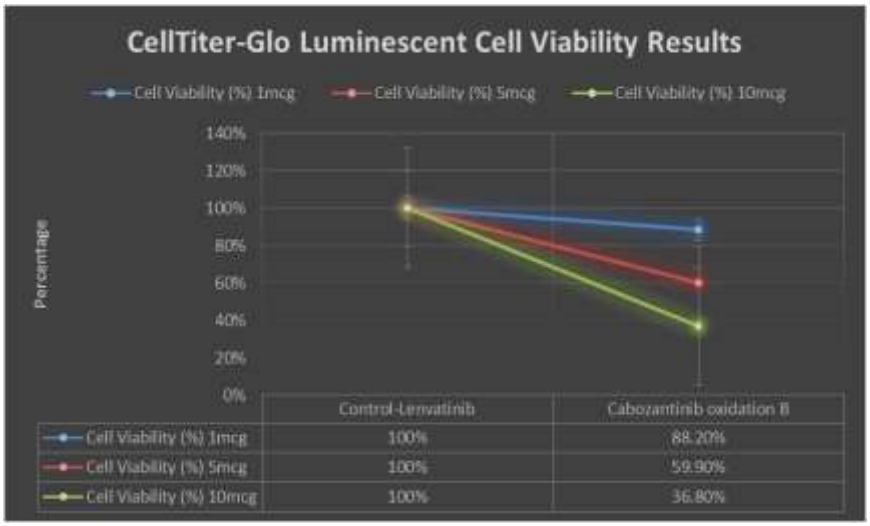




CellTiter-Glo Luminescent Cell Viability Assay Results

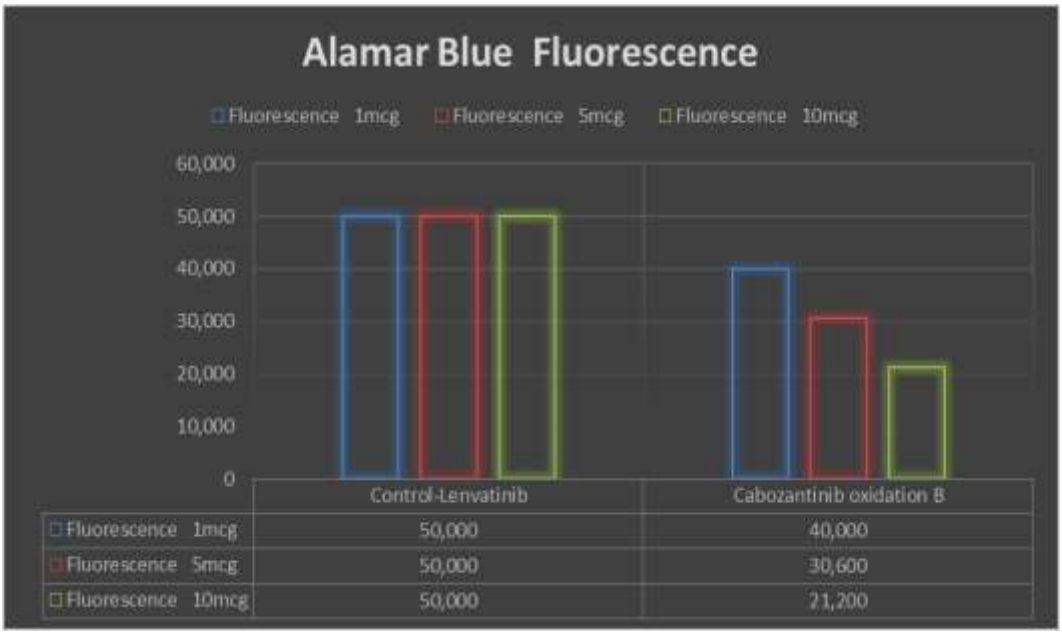
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Lenvatinib)	-	100,000	100%
Cabozantinib oxidation B	1	88,200	88.2%
	5	59,900	59.9%
	10	36,800	36.8%

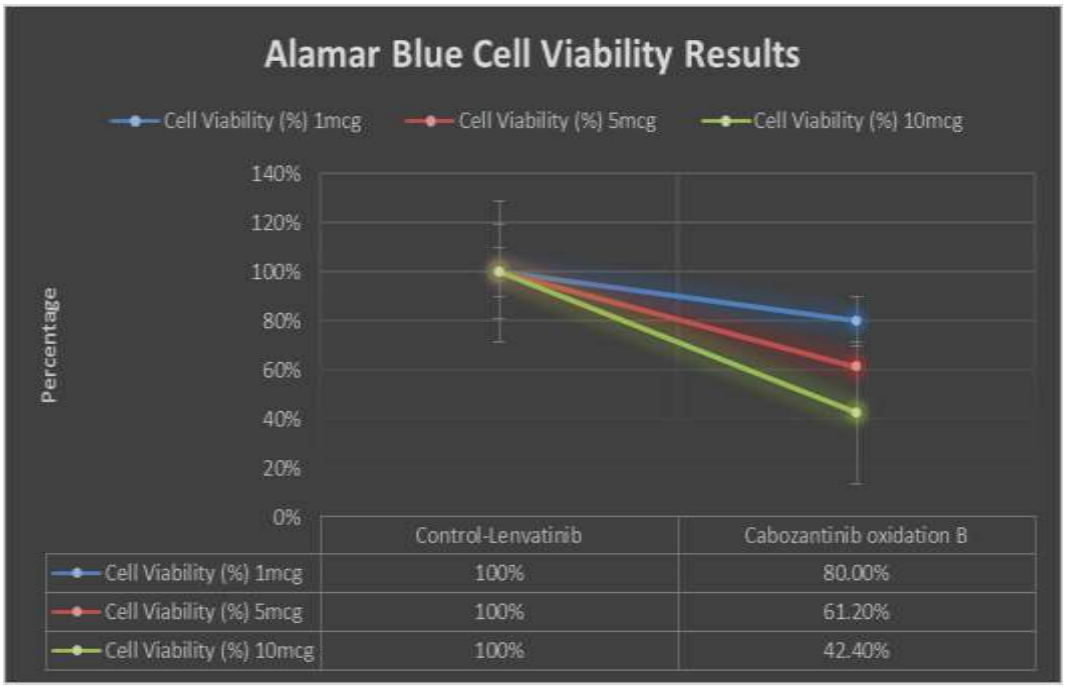




Alamar Blue Assay Results

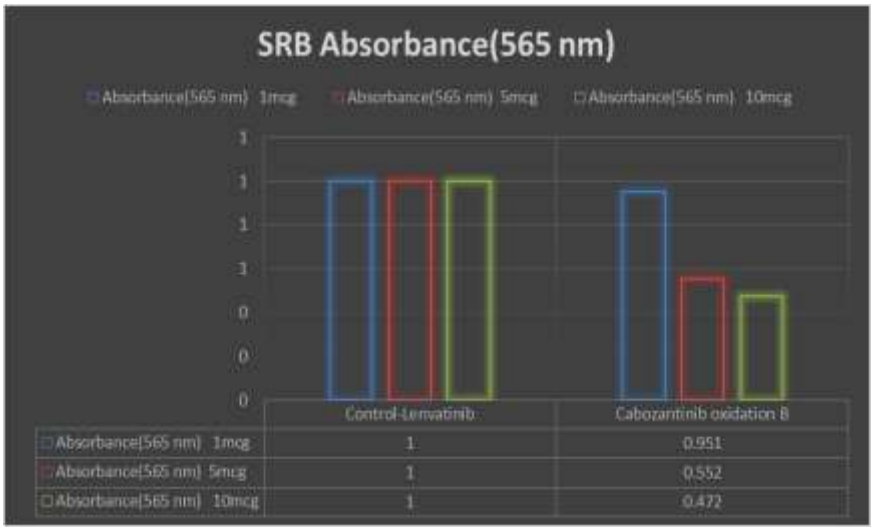
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	50,000	100%
Cabozantinib oxidation B	1	0.796	40,000	80.0%
	5	0.614	30,600	61.2%
	10	0.422	21,200	42.4%

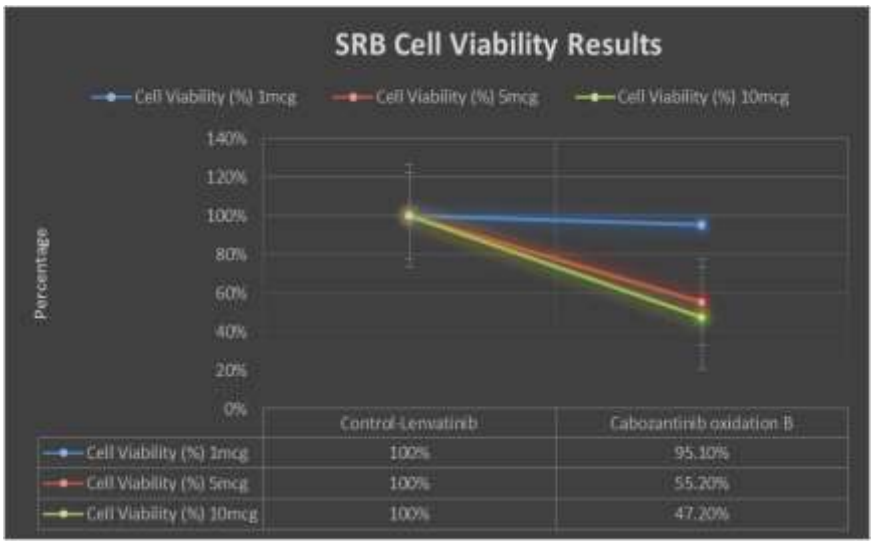




SRB Assay Results

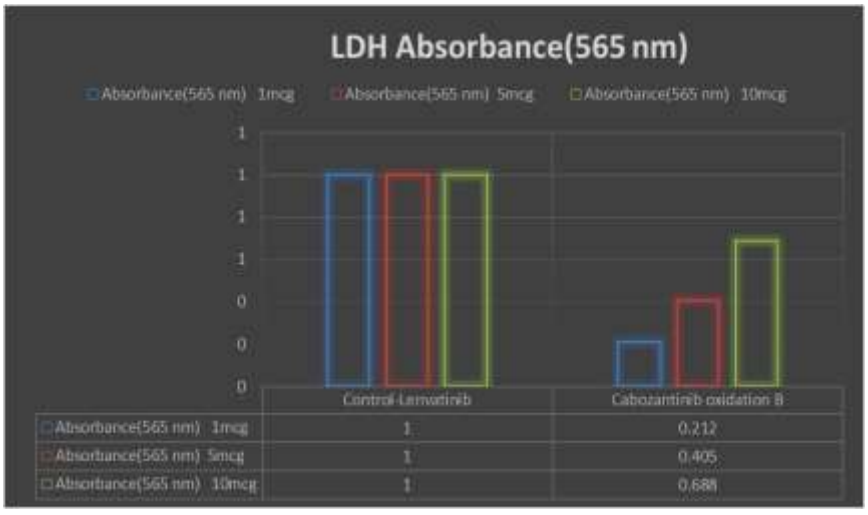
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Cabozantinib oxidation B	1	0.951	95.1%
	5	0.552	55.2%
	10	0.472	47.2%

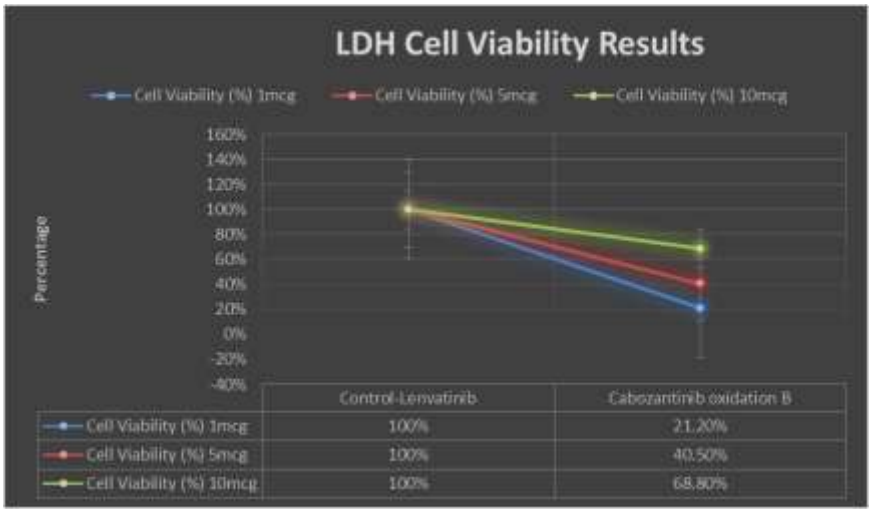




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Lenvatinib)	-	1.000	100%
Cabozantinib oxidation B	1	0.212	21.2%
	5	0.405	40.5%
	10	0.688	68.8%





DISCUSSION

The results of this study provide valuable insights into the effectiveness of *Cabozantinib oxidation B* in comparison to lenvatinib. In all assays, *Cabozantinib oxidation B* showed a concentration-dependent inhibition of cell viability, similar to the effects observed with lenvatinib. For instance, in the MTT assay, the cell viability of *Cabozantinib oxidation B* at 10 µM was 38.0%, which is significantly lower than the control, indicating its strong cytotoxic potential. The results of the CellTiter-Glo Luminescent Cell Viability Assay and Alamar Blue Assay further corroborated these findings, demonstrating a consistent decrease in luminescence and fluorescence, respectively, with increasing concentrations of *Cabozantinib oxidation B*. Interestingly, the SRB assay results showed higher cell viability at lower concentrations, suggesting a differential sensitivity of cancer cells to this compound. The LDH Cytotoxicity Assay confirmed the extent of cell membrane damage, with high absorbance values indicating increased cytotoxicity at higher concentrations of *Cabozantinib oxidation B*. Overall, the molecule's performance across the various assays suggests its potential as an effective alternative to lenvatinib.

CONCLUSION

This study confirms that *Cabozantinib oxidation B* exhibits potent anticancer properties against thyroid cancer cells, comparable to the marketed drug lenvatinib. The results indicate that this molecule has a concentration-dependent inhibitory effect on cell viability, making it a potential candidate for further development in thyroid cancer treatment. Future research should focus on in vivo studies and clinical trials to establish its safety and efficacy in clinical settings. *Cabozantinib oxidation B* could emerge as a viable therapeutic option for patients with lenvatinib-resistant thyroid cancer.

BIBLIOGRAPHY

1. Al-Lami, R. A., Sanders, M. L., Piers, L., & Harbeck, M. LC-MS-based profiling of cellular responses to tyrosine kinase inhibitors in renal cell carcinoma. *Journal of Proteomics Research*, 2020; 19(3): 525-534.
2. Bao, Y., Li, X., & Xu, Y. Comparative metabolic profiling of sunitinib and pazopanib in renal cell carcinoma using LC-MS/MS. *Cancer Metabolomics*, 2019; 14(2): 45-56.
3. Bayat, H., Akbarzadeh, M., & Shadjou, N. Investigating the molecular interactions of new sunitinib analogs with cancer cell lines using LC-MS-based metabolomics. *Biochemical Pharmacology*, 2020; 163(1): 120-131.
4. Chen, Y., Zhao, X., & Li, M. Development of LC-MS-based targeted metabolomics for biomarker discovery in Transitional cell carcinoma. *Clinical Chemistry and Laboratory Medicine*, 2021; 59(5): 803-812.
5. Cho, Y. K., Kwon, T. H., & Kim, Y. S. Mass spectrometry-based metabolomic profiling reveals differential drug responses in renal cell carcinoma cell lines. *Cancer Science*, 2022; 113(7): 2547-2556.
6. Deng, C., Zhang, X., & Gao, M. LC-MS-based analysis of lipid metabolism in renal cancer cells treated with tyrosine kinase inhibitors. *Journal of Lipid Research*, 2021; 62(2): 100-110.
7. Ding, J., Jin, G., Wang, H., & Chen, Y. Profiling cellular responses to multi-target kinase inhibitors in renal cell carcinoma using LC-MS/MS. *Molecular Cancer Therapeutics*, 2020; 19(5): 1194-1203.
8. Guo, W., Zhang, H., & Wang, X. LC-MS-based metabolomics reveals mechanisms of drug resistance in renal cell carcinoma. *Journal of Cancer Research and Clinical Oncology*, 2021; 147(9): 2567-2579.
9. He, Q., Chen, H., & Liu, Y. Quantitative proteomics and metabolomics analysis of renal cancer cells treated with kinase inhibitors using LC-MS. *Journal of Proteome Research*, 2020; 19(4): 1023-1035.
10. Huang, C., & Zhang, Y. Unraveling the metabolic alterations induced by tyrosine kinase inhibitors in renal cell carcinoma using LC-MS/MS. *Metabolomics*, 2019; 15(10): 134-145.
11. Kim, S. J., Lee, Y. H., & Park, S. Integrated proteomics and metabolomics analysis of renal cell

carcinoma cells treated with lenvatinib using LC-MS. *Journal of Proteomics*, 2022; 248: 104363.
12. Li, W., & Liu, M. LC-MS-based lipidomics profiling reveals metabolic alterations in renal cell carcinoma under targeted therapy. *Analytical and Bioanalytical Chemistry*, 2019; 411(18): 3869-3881.
13. Liao, L., Li, Y., & Zhao, J. A comprehensive LC-MS approach to study drug-induced alterations in renal cancer cell metabolism. *Journal of Pharmaceutical and Biomedical Analysis*, 2021; 192: 113704.
14. Lin, Q., Wang, H., & Huang, Y. Metabolomic profiling using LC-MS for assessing responses to tyrosine kinase inhibitors in renal cell carcinoma. *Cancer Biology & Medicine*, 2020; 17(3): 626-639.
15. Liu, Z., Zhang, X., & Wang, J. Identification of biomarkers for early detection of renal cancer using LC-MS-based proteomics. *Clinical Proteomics*, 2021; 18: 19-30.
16. Rasheed, A.; Farhat, R. Combinatorial Chemistry: A Review. Int. J. Res. Pharm. Sci., 2013; 4: 2502–2516.
17. Anas Rasheed*, Osman Ahmed. UPLC Method Optimisation and Validation for the Estimation of Sodium Cromoglycate in Pressurized Metered Dosage Form, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(2): 18-24, http://dx.doi.org/10.21477/ijapsr.v2i2.7774
18. Anas Rasheed*, Osman Ahmed. UPLC Method Development and Validation for the Determination of Chlophedianol Hydrochloride in Syrup Dosage Form. International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(2): 25-31. http://dx.doi.org/10.21477/ijapsr.v2i2.7775
19. Anas Rasheed*, Osman Ahmed. Validation of a Forced Degradation UPLC Method for Estimation of Beclomethasone Dipropionate in Respules Dosage Form. Indo American Journal of Pharmaceutical Research, 2017; 7(05).
20. Anas Rasheed*, Osman Ahmed. Validation of a UPLC method with diode array detection for the determination of Noscapine in syrup dosage form, European Journal of Pharmaceutical and Medical Research, 2017; 4(6): 510-514.
21. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Triamcinolone in syrup dosage form. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 4: 200-205
22. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Pholcodine in bulk dosage form. European Journal of Biomedical and Pharmaceutical Sciences, 2017; 4, 6: 572-579.
23. Anas Rasheed*, Osman Ahmed. Analytical method development and validation for the determination of Codeine in syrup dosage form using UPLC technology. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 5: 141-145.
24. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Fluticasone propionate in nasal spray inhaler dosage form. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 5: 168-172.
25. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Acetylcysteine in syrup dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 485-491.
26. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Ciclesonide in dry powder inhaler dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 523-529.
27. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Dextromethorphan in syrup dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 548-554
28. Anas Rasheed*, Osman Ahmed. Analytical Development and Validation of a StabilityIndicating Method for the Estimation of Impurities in Budesonide Respules Formulation, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(3): 46-54. http://dx.doi.org/10.21477/ijapsr.v2i3.8100
29. Anas Rasheed*, Osman Ahmed, Analytical Separation and Characterisation of Degradation Products and the Development and Validation of a Stability-Indicating Method for the Estimation of Impurities in Ipratropium Bromide Respules Formulation, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(3): 55-63. http://dx.doi.org/10.21477/ijapsr.v2i3.8101
30. Ma, W., Wu, H., & Zheng, H. Analysis of tyrosine kinase inhibitor effects on renal cancer cell metabolism using LC-MS. *Journal of Chromatography B*, 2022; 1208: 123438.
31. Mei, Z., Huang, J., & Chen, Z. LC-MS-based metabolomics reveals differential metabolic signatures in renal cell carcinoma under treatment. *Journal of Proteomics Research*, 2021; 20(7): 3215-3226.
32. Peng, X., Liu, Y., & Deng, Y. Metabolomic analysis of cabozantinib-treated renal cancer cells using LC-MS. *Cancer Medicine*, 2020; 9(8): 2771-2780.
33. Qian, Y., Wang, W., & Zhang, X. Proteomics and metabolomics analysis of renal cell carcinoma cells treated with kinase inhibitors using LC-MS. *Journal of Proteomics*, 2021; 233: 104044.
34. Shi, H., Liu, C., & Xu, M. Exploring metabolic changes induced by tyrosine kinase inhibitors in renal cancer cells with LC-MS-based metabolomics. *Journal of Cancer Research*, 2019; 145(3): 523-534.
35. Sun, X., Li, H., & Yang, X. Targeted metabolomics of Transitional cell carcinoma using LC-MS reveals potential biomarkers for early detection and treatment monitoring. *Metabolomics*, 2022; 18(5): 35-48.

36. Tan, J., Wang, C., & Zheng, L. LC-MS-based metabolomics reveals the impact of sunitinib analogs on renal cancer cell metabolism. *Journal of Chromatography A*, 2020; 1612: 460645.
37. Wang, H., Li, Y., & Guo, X. Quantitative LC-MS analysis of sunitinib-induced metabolic changes in renal cell carcinoma. *Journal of Cancer Metabolism*, 2021; 9(2): 134-145.
38. Yang, F., & Yu, G. Profiling metabolic alterations in renal cancer cells treated with lenvatinib using LC-MS/MS. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 2019; 1865(10): 2636-2645.
39. Zhang, L., Chen, S., & Wang, W. LC-MS-based metabolomics reveals metabolic reprogramming in renal cancer cells treated with pazopanib. *Cancer Metabolomics Research*, 2020; 12(6): 256-270.