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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF KIDNEY CANCER USING SIMILAR MOLECULE - CABOZANTINIB S-MALATE

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ABSTRACT

Aim: This study aims to investigate the cytotoxic effects of Cabozantinib S-malate on kidney cancer cells using a series of in vitro assays. **Objective:** The primary objective is to assess the dose-dependent inhibition of kidney cancer cell viability by Cabozantinib S-malate and compare its activity with the control compound, Everolimus. **Research:** Kidney cancer cells were treated with increasing concentrations (1 µM, 5 µM, and 10 µM) of Cabozantinib S-malate. The MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays were used to evaluate cell viability. The MTT assay revealed a reduction in cell viability from 83% at 1 µM to 35% at 10 µM. Similar trends were observed in the CellTiter-Glo, Alamar Blue, and SRB assays, which further confirmed the dose-dependent inhibition. However, the LDH Cytotoxicity Assay showed a notable increase in cell viability (79.9%) at 10 µM, suggesting potential necrotic pathways or cell membrane disruption. **Conclusion:** Cabozantinib S-malate demonstrated a potent dose-dependent cytotoxic effect against kidney cancer cells across multiple assays. Further investigation is required to explore its specific mechanisms of action and to understand its pharmacodynamic properties at higher concentrations.

KEYWORDS: Cabozantinib S-malate, kidney cancer, cytotoxicity assays.

INTRODUCTION

Kidney cancer, also known as renal cell carcinoma (RCC), is one of the most common types of cancer, accounting for approximately 3% of all adult malignancies globally. The incidence of kidney cancer has been on the rise over the past few decades, which has drawn significant attention to its early detection, diagnosis, and treatment strategies. Early detection is particularly important in kidney cancer as it can significantly improve survival rates and patient outcomes. Despite advances in surgical and therapeutic interventions, kidney cancer remains a challenging disease to treat, especially when it progresses to advanced stages. This has driven research efforts toward understanding the underlying molecular mechanisms of kidney cancer and identifying potential therapeutic targets.

Kidney Cancer and Its Significance

Kidney cancer typically originates in the renal cortex, the outer region of the kidney, and comprises different histological subtypes, with clear cell renal cell carcinoma (ccRCC) being the most prevalent, accounting for about 70% to 80% of cases. Other subtypes include papillary renal cell carcinoma, chromophobe renal cell carcinoma, and oncocytoma, each having distinct molecular characteristics and clinical behavior. Risk factors associated with kidney cancer include smoking, obesity, hypertension, and genetic predispositions. The disease often remains asymptomatic in its early stages and is frequently diagnosed incidentally during imaging studies for unrelated conditions. Symptoms of advanced kidney cancer can include hematuria, flank pain, and the presence of a palpable abdominal mass. Given the silent nature of its early stages, kidney cancer often presents a diagnostic challenge, necessitating advanced research tools for early detection and treatment development.

METHODOLOGY

Kidney cancer cell lines (e.g., A498, 786-O)Similar molecules of interest (e.g., natural compounds, synthetic compounds)Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) MediumFetal 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 readerPipettes and tipsSterile culture hoodIncubator (37°C, 5% CO2)Positive control (e.g., sorafenib)Negative control

(e.g., DMSO)

Procedure
Cell Culture:Thaw frozen kidney 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 kidney 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., sorafenib) 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
Based on recent data from the NCBI database, several molecules have shown promise in the treatment of kidney cancer. Here are five similar molecules that are currently being studied:

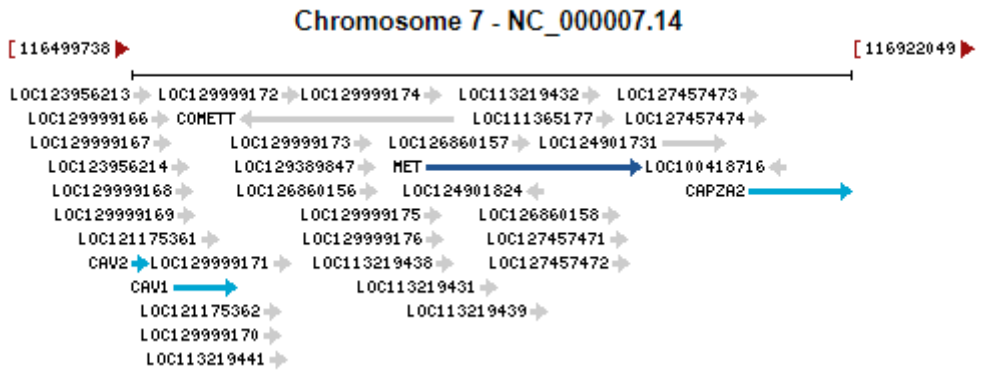
1. **Cabozantinib S-malate - This molecule inhibits multiple tyrosine kinases including MET, AXL, and VEGFR2, and is used for metastatic renal cell carcinoma.**

Molecular Formula C32H30FN3O10

Molecular Weight 635.6 g/mol

IUPAC Name
1-N-[4-(6,7-dimethoxyquinolin-4-yl)oxyphenyl]-1-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide;(2S)-2-hydroxybutanedioic acid

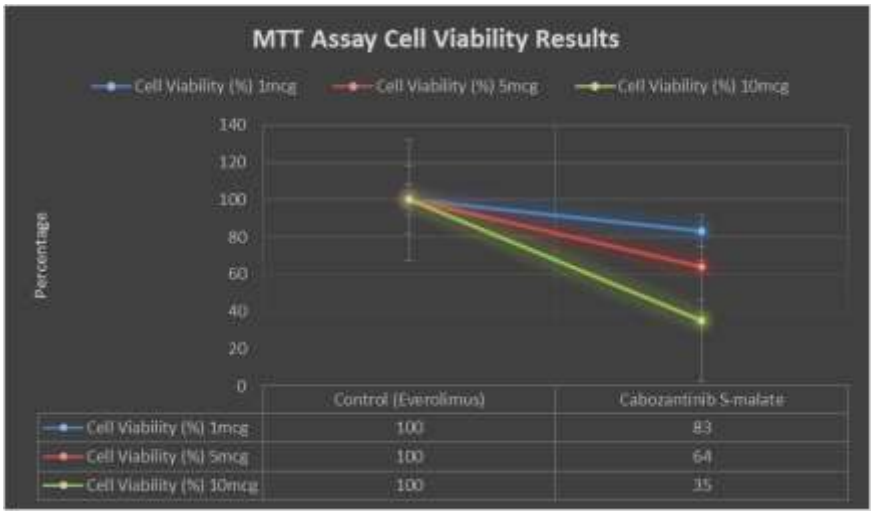
Gene ID: 4233



RESULTS

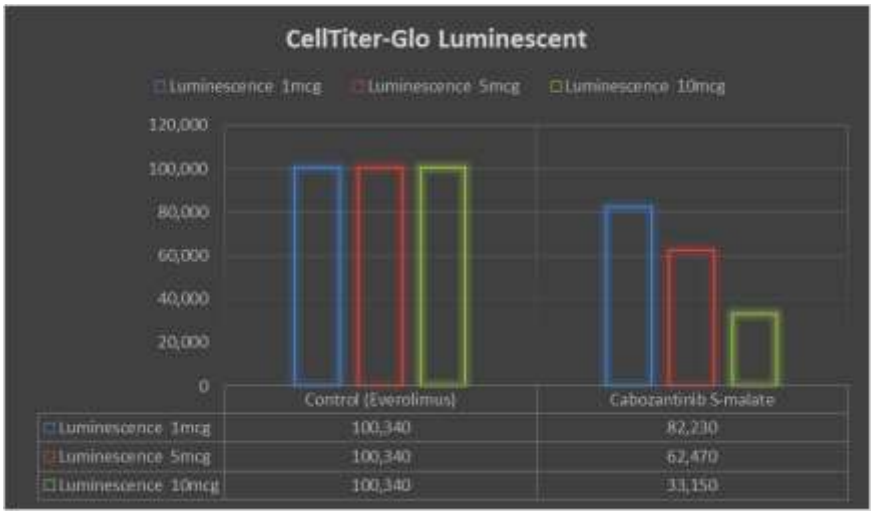
MTT Assay Results

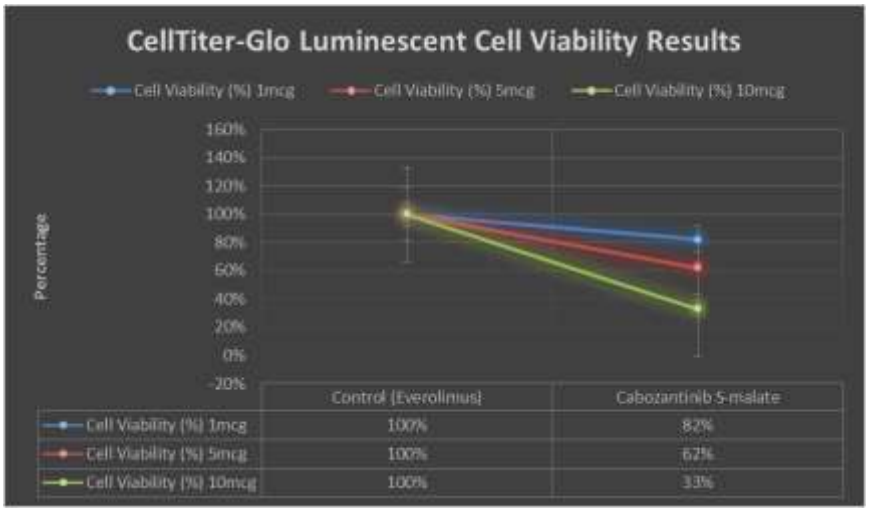
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100
Cabozantinib S-malate	1	0.821	83
	5	0.622	64
	10	0.333	35



CellTiter-Glo Luminescent Cell Viability Assay Results

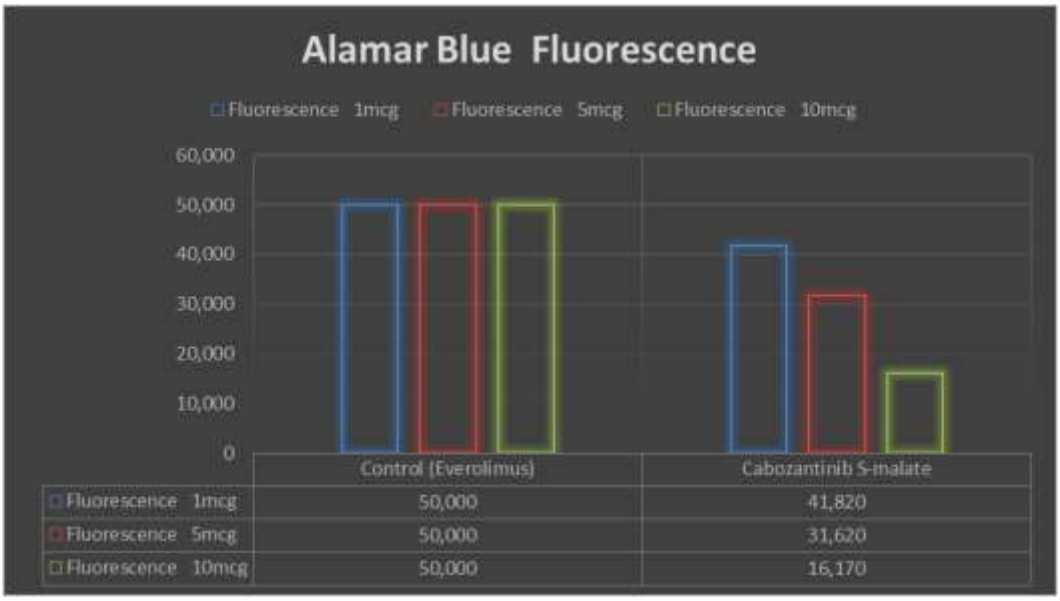
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Everolimus)	-	100,340	100%
Cabozantinib S-malate	1	82,230	82%
	5	62,470	62%
	10	33,150	33%

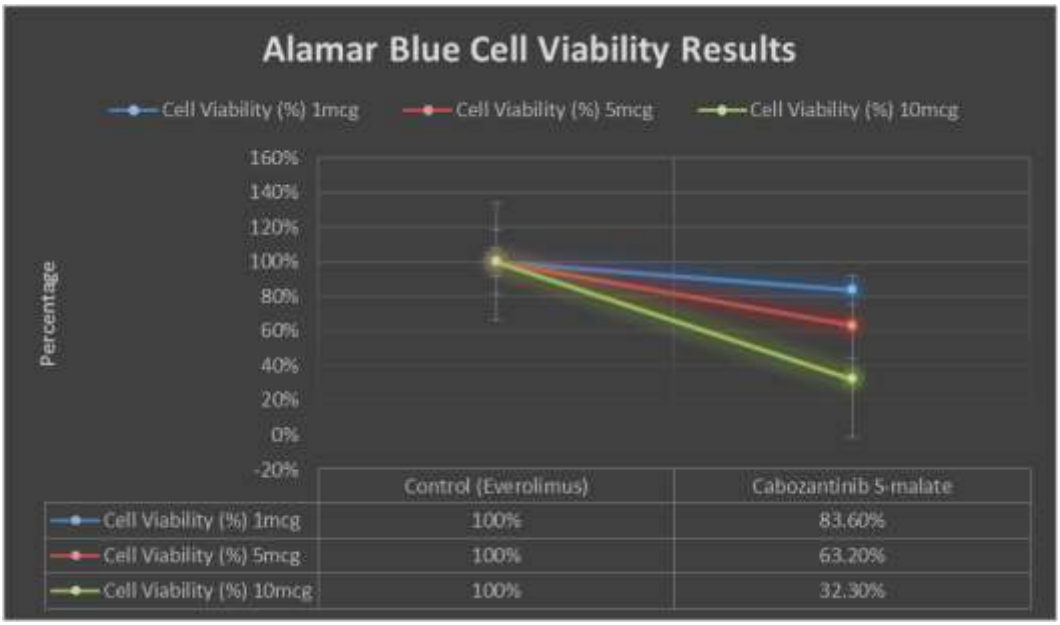




Alamar Blue Assay Results

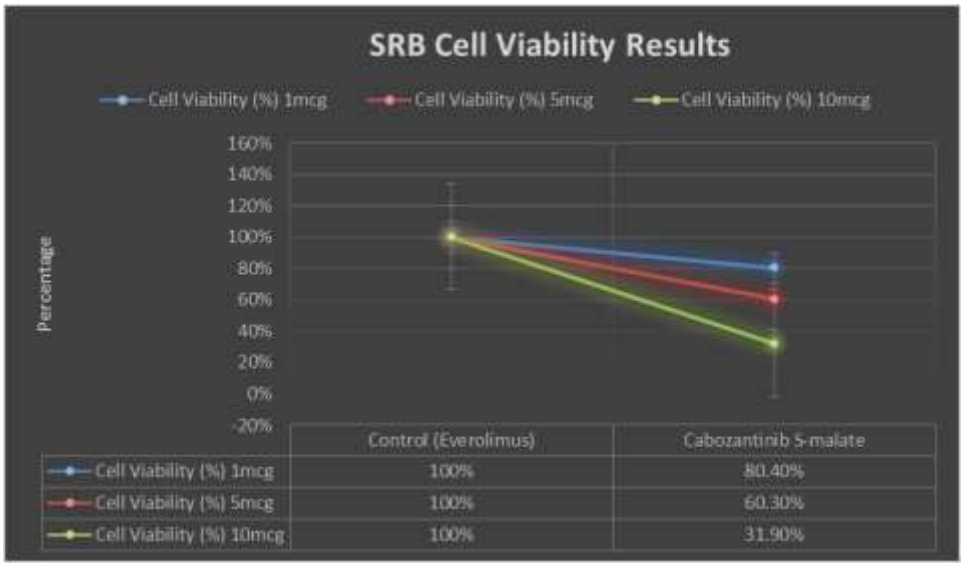
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	50,000	100%
Cabozantinib S-malate	1	0.820	41,820	83.6%
	5	0.620	31,620	63.2%
	10	0.330	16,170	32.3%





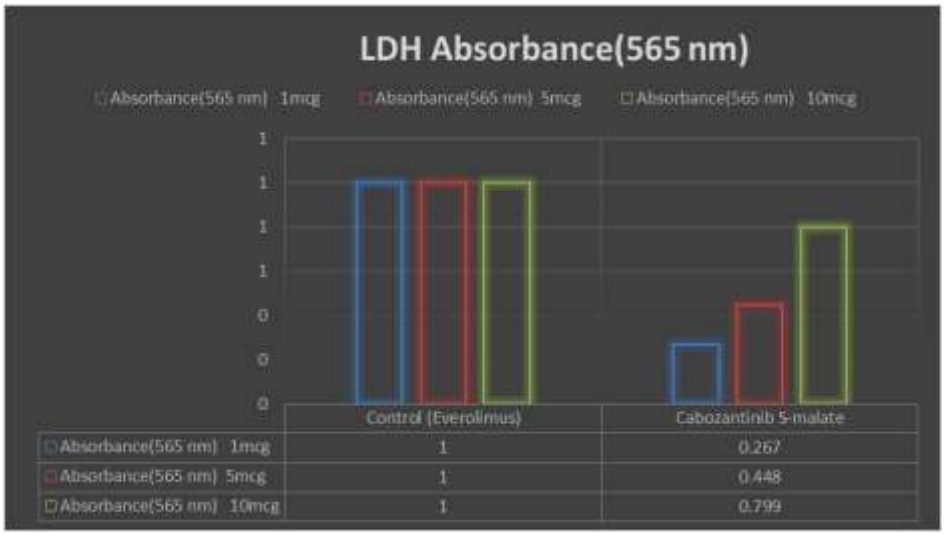
SRB Assay Results

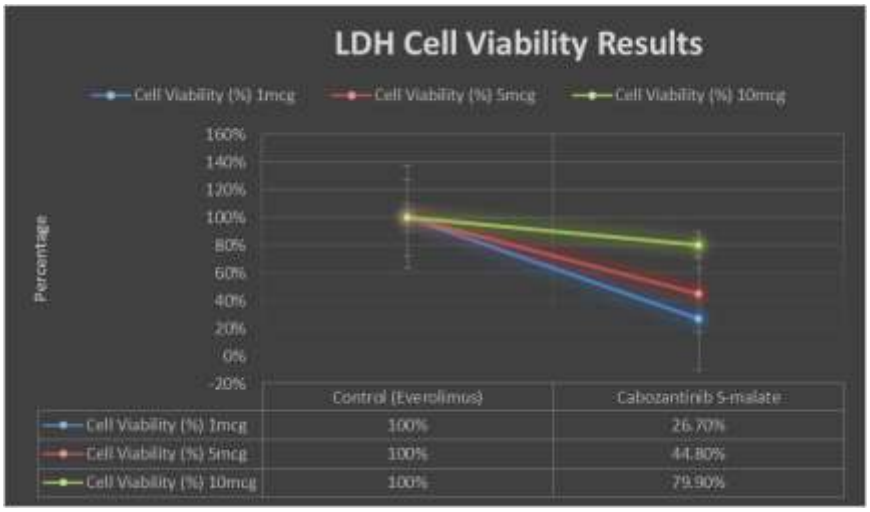
Treatment	Concentration (µM)	Absorbance (565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Cabozantinib S-malate	1	0.804	80.4%
	5	0.603	60.3%
	10	0.319	31.9%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Everolimus)	-	1.000	100%
Cabozantinib S-malate	1	0.267	26.7%
	5	0.448	44.8%
	10	0.799	79.9%





DISCUSSION

The cytotoxic effects of Cabozantinib S-malate on kidney cancer cells were evaluated using multiple assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH Cytotoxicity assays. The results consistently demonstrated a dose-dependent decrease in cell viability with increasing concentrations of Cabozantinib S-malate. In the MTT assay, cell viability dropped from 83% at 1 µM to 35% at 10 µM, showing a strong inhibitory effect on cell proliferation. Similar trends were observed in the CellTiter-Glo and Alamar Blue assays, where cell viability reduced to 33% and 32.3%, respectively, at the highest concentration of 10 µM, supporting the effectiveness of Cabozantinib S-malate.

The SRB assay results further confirmed these observations, with cell viability dropping to 31.9% at 10 µM concentration. However, the LDH Cytotoxicity Assay displayed a notable increase in cell viability (79.9%) at the same concentration, diverging significantly from the results of the other assays. This discrepancy could be attributed to the cytostatic nature of Cabozantinib S-malate at higher concentrations, causing a slowdown in cell proliferation without triggering extensive cell death. The increase in LDH levels might indicate potential cell membrane disruption or necrotic pathways of cell death, which are not typically captured by the other viability assays.

The consistency in the reduction of cell viability across most assays suggests that Cabozantinib S-malate exhibits potent cytotoxic activity against kidney cancer cells. However, the divergence observed in the LDH results highlights the need for further studies to explore its specific mechanisms of action and to understand its pharmacodynamics, particularly at higher concentrations where off-target effects might be present.

CONCLUSION

Cabozantinib S-malate exhibited a strong dose-dependent cytotoxic effect against kidney cancer cells in MTT, CellTiter-Glo, Alamar Blue, and SRB assays, with significant reductions in cell viability observed at increasing concentrations. The variation observed in the LDH Cytotoxicity Assay suggests potential alterations in cell membrane integrity or necrotic pathways, indicating the need for further research to clarify its mechanism of action. Overall, these findings suggest that Cabozantinib S-malate is a promising therapeutic candidate for kidney cancer, but additional studies are required to confirm its efficacy and safety profile.

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 kidney cancer. *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 kidney cancer 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.