International Journal of Drug Research in Clinics

Int J Drug Res Clin 2026;4:e9101
doi:10.34172/ijdrc.9101
http://ijdrug.com

Original Article

Imipramine Sensitizes Glioblastoma Cells to Temozolomide via PI3K/Akt Inhibition and Mitochondrial Apoptosis Induction

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Article History:
Received: September 29, 2025
Revised: January 22, 2026
Accepted: February 21, 2026
ePublished: April 13, 2026

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Abstract

Introduction: Glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor, is characterized by a poor prognosis, with a median survival of only 12-18 months. This unfavorable outcome is largely attributed to severe resistance to temozolomide (TMZ), the standard chemotherapeutic agent used for treatment, which leads to treatment failure and tumor recurrence. Imipramine, a tricyclic antidepressant, has demonstrated potential as a modulator of signaling pathways. This study aimed to investigate the role of imipramine in increasing the sensitivity of U87MG glioblastoma cells to TMZ through the inhibition of the PI3K/Akt pathway.

Methods: In this experimental study, U87MG cells were treated with TMZ and imipramine, both as single agents and in combination. Cell survival and proliferation were assessed using the MTT assay. Gene expression levels of PI3K, Akt, Bax, Bcl-2, and Caspase-3 were measured via quantitative real-time polymerase chain reaction (qRT-PCR). Apoptosis rates were also measured using enzyme-linked immunosorbent assay (ELISA) and flow cytometry.

Results: Individually, TMZ and imipramine exerted inhibitory effects on cell proliferation. However, their combination significantly reduced the TMZ IC50 value from 78 to 35 μM. Combination treatment led to a significant downregulation of PI3K and Akt gene expression accompanied by upregulation of Bax and Caspase-3 and downregulation of Bcl-2 ($P<0.05$). The apoptosis rate in the combination group exceeded 82%, and the combination with a PI3K inhibitor increased this rate to 94%.

Conclusion: These results indicate that imipramine can effectively reverse intrinsic glioblastoma resistance to TMZ by concurrently inhibiting the PI3K/Akt pathway and activating the mitochondrial apoptosis pathway. Given the established safety profile of imipramine, its potential use as an adjuvant drug in the treatment of glioblastoma is significant and requires further investigation through animal models and subsequent clinical trials.

Keywords: Glioblastoma, Imipramine, Temozolomide, PI3K/Akt pathway, Apoptosis

Please cite this article as follows: Bazazzadeh N, Molavand M, Yousefi B, Shimia M. Imipramine sensitizes glioblastoma cells to temozolomide via PI3K/Akt inhibition and mitochondrial apoptosis induction. Int J Drug Res Clin 2026;4:e9101. doi:10.34172/ijdrc.9101

Introduction

Glioma is one of the most prevalent and aggressive tumors of the central nervous system (CNS).[1,2] The World Health Organization (WHO) classifies malignant gliomas into two grades: grade III tumors, encompassing anaplastic ependymomas, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, and anaplastic astrocytoma.[3,4] Grade IV tumors are classified as glioblastoma. This WHO classification is based on nuclear atypia, mitotic activity, vascular proliferation, necrosis, proliferative potential, clinical course, and treatment outcome.[5] Glioblastoma is the most frequent CNS malignancy, accounting for 45.2 % of all CNS tumors and 80% of primary malignant CNS tumors. Its incidence rate is significantly higher in women and individuals of white ethnicity.[6] The gold standard treatment for patients with glioma is surgery followed by chemoradiation.[7] Temozolomide (TMZ), an orally administered alkylating agent, is the most common chemotherapeutic agent for treating glioma patients.[8] Despite these interventions, current therapeutic modalities fail to increase the overall survival of patients with gliomas, with a median survival of only 12–18 months for newly

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diagnosed glioblastoma patients.[9,10] One important reason for the failure of chemotherapy to improve overall survival in glioma patients is the development of drug resistance, particularly to TMZ. This underscores the urgent need to identify novel biomarkers involved in glioma progression and to develop more effective therapeutic modalities for patients with gliomas.[11]

TMZ is used as the drug of choice in glioblastoma multiforme (GBM) treatment protocols. It inhibits replication and ultimately causes cell death by inducing methylation of guanine bases within the DNA.[12] However, a significant proportion of patients exhibit a decrease in TMZ efficacy over time, which is mainly attributed to the activation of drug resistance mechanisms. Key contributors to this phenomenon include the overexpression of the enzyme O6-methylguanine-DNA methyltransferase (MGMT), the activation of DNA repair pathways such as Mismatch Repair (MMR) and Base Excision Repair (BER), epigenetic alterations, and the dysregulation of survival signaling pathways.[13] These mechanisms collectively reduce the cellular response to TMZ, leading to tumor recurrence. Consequently, identifying strategies to increase GBM cell sensitivity to TMZ is of particular clinical importance.

Hyperactivity of the PI3K/Akt pathway, observed in over 70% of GBM cases, plays a crucial role in resistance to TMZ.[14] Activation of this pathway leads to inhibition of apoptosis, increased cell proliferation, and enhanced DNA repair mechanisms.[15] Therefore, inhibiting this pathway can be an effective strategy for increasing the sensitivity of tumor cells to TMZ.

The Potential of Imipramine in Cancer Treatment

Imipramine, a tricyclic antidepressant, has recently attracted attention for its anticancer effects in preclinical studies.[16] This drug acts through multiple mechanisms, including inhibition of the PI3K/Akt/mTOR pathway, leading to decreased phosphorylation of key pathway proteins, induction of apoptosis through caspase activation and impaired mitochondrial function, decreased expression of antiapoptotic factors (e.g., Bcl-2), and increased expression of proapoptotic proteins (e.g., Bax).[17] In vitro studies have indicated that imipramine can enhance the sensitivity of cancer cells to chemotherapeutic drugs. For example, a study on breast cancer cells demonstrated that the combination of imipramine and doxorubicin significantly decreased tumor cell survival.[18] However, the effect of imipramine on GBM cells, especially in combination with TMZ, has not been comprehensively investigated. Although several studies have confirmed the role of the PI3K/Akt pathway in TMZ resistance and highlighted the anticancer effects of imipramine,[19] no study has systematically investigated the effect of imipramine on the sensitization of GBM cells to TMZ through modulation of the PI3K/Akt pathway.

To validate the role of the PI3K/Akt pathway in mediating these effects, we employed LY294002, a well-characterized selective PI3K inhibitor that blocks PI3K activity by competing with ATP at the catalytic site. This pharmacological tool has been extensively used to dissect PI3K-dependent cellular processes and serves as a benchmark for assessing pathway-specific inhibition.

This study was designed to investigate the effect of imipramine on sensitizing glioblastoma cells to TMZ through inhibition of the PI3K/Akt pathway. The findings can provide a foundation for developing novel combination therapeutic regimens and improving treatment responses in GBM patients. Furthermore, given imipramine's well-known clinical safety profile, if proven effective, it may serve as an adjunct within GBM treatment protocols.

Methods

Cell Culture

Human glioblastoma U87MG cells (Pasteur Institute of Iran Cell Bank) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37˚C in a humidified incubator with 5% CO_2. The culture medium was changed every 2–3 days, and cells were detached and passaged at 70–80% confluence using 0.25% trypsin.

MTT Assay

Cell viability after treatment with drugs was determined using the MTT assay. U87MG cells were seeded into 96-well plates at a density of 5000 cells per well and incubated for 24 hours to allow cell adhesion. Subsequently, cells were treated with different doses of TMZ, imipramine, or their simultaneous combination for 48 hours. Then, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, and plates were incubated for 4 hours. Thereafter, the medium was removed from the wells, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The optical absorbance was measured at 570 nm using an Enzyme-Linked Immunosorbent Assay (ELISA) reader. The percentage of cell survival was calculated compared to the control group.

The 48-hour treatment period was selected based on preliminary time-course experiments (data not shown) and on previous literature demonstrating that this duration is sufficient for drug uptake, metabolic processing, and the development of cytotoxic effects while maintaining appropriate cell viability in control groups. This regimen is consistent with standard protocols for TMZ treatment in glioblastoma cell line studies.

Quantitative Real-Time Polymerase Chain Reaction

To evaluate changes in gene expression after treatment, total RNA was extracted from the cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA purity and concentration were checked using a NanoDrop spectrophotometer, and samples with an A260/A280 absorbance ratio of 1.8-2 were used for Complementary DNA (cDNA) synthesis. cDNA was synthesized using a cDNA synthesis kit (Parstous).

The quantitative real-time polymerase chain reaction (qRT-PCR) reaction was performed using a master mix containing SYBR Green (Parstous) and gene-specific primers for Bax, Bcl-2, caspase-3, PI3K, and AKT. β-actin was used as the internal reference gene. Table 1 lists all the primer sequences for the genes analyzed in this study. The thermal conditions of the reaction included initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. Data analysis was performed using the $2^{-\Delta\Delta Ct}$ method.

Measurement of Caspase-3/7 Activity

After drug treatment, caspase-3/7 activity in U87MG cells was measured using a fluorometric kit according to the manufacturer's instructions. Cells were incubated with fluorogenic substrate DEVD-AFC. After 30–60 minutes of incubation in the dark, fluorescence intensity was measured using a microplate reader with an excitation wavelength of 400 nm and an emission wavelength of 505 nm. The results were analyzed relative to the control group.

Enzyme-Linked Immunosorbent Assay-Based Apoptosis Detection

Apoptosis was assessed using a cell death detection ELISA kit (e.g., Roche Diagnostics), which detects apoptosis quantitatively. After treatment, 20 µL of the supernatant was transferred to a 96-well plate pre-coated with anti-histone antibodies. Following incubation and washing steps, an anti-DNA-peroxidase conjugate was added. Utilizing a microplate reader, the absorbance of the chromogenic substrate was measured at 405 nm.

Statistical Analysis

All experiments were performed at least in triplicate, and results are presented as mean ± standard deviation (SD). Data were analyzed using GraphPad Prism software (version 16). One-way ANOVA followed by Tukey's multiple comparison test was used to assess the statistical significance among groups. A $P < 0.05$ was considered statistically significant in all analyses.

Results

Imipramine Enhances the Cytotoxic Effect of Temozolomide in U87MG Cells

Cell viability assay revealed that TMZ decreased U87MG cell survival in dose-depended manner, with an IC_{50} value of 78 µM (Figure 1). Imipramine also showed dose-dependent cytotoxicity, with an IC_{50} value of 181 µM (Figure 1). Additionally, the combination of imipramine with varying concentrations of TMZ in U87MG significantly reduced the IC50 value to 35 µM, indicating a synergistic effect in reducing the growth of cancer cells and increasing their sensitivity to TMZ.

Combination Treatment Inhibits PI3K/AKT Signaling and Modulates Apoptotic Gene Expression in U87MG Cells

The effects of imipramine on PI3K mRNA in U87MG cells were assessed using qRT-PCR. PI3K expression levels were significantly decreased in U87MG cells treated with TMZ and imipramine alone compared to the control group ($P < 0.05$; Figure 2). The combined treatment resulted in an even greater decrease in PI3K expression compared to cells treated with TMZ and imipramine alone ($P < 0.05$). Similarly, AKT expression was significantly reduced in U87MG cells treated with TMZ and imipramine alone compared to the control group ($P < 0.05$; Figure 2), with a greater decrease in the combination group compared to cells treated with TMZ and imipramine alone ($P < 0.05$). Moreover, treatment with LY294002 in combination with TMZ and imipramine caused a further decrease in the expression of both genes ($P < 0.05$).

Treatment with TMZ or imipramine alone reduced PI3K and AKT expression, whereas combined treatment led to a more pronounced decrease in U87MG cells. Co-treatment with LY294002, TMZ, and imipramine showed an additional reduction in PI3K and AKT expression ($P < 0.05$). Data are presented as mean ± SD of three independent experiments.

Furthermore, the effects of imipramine on the mRNA expression of apoptotic genes (Bax, Bcl-2, and caspase-3) in U87MG cells were investigated by qRT-PCR. The expression levels of Bax and caspase-3 (pro-apoptotic genes) were significantly higher in U87MG cells treated with TMZ and imipramine alone than in the control group ($P < 0.05$; Figure 3). Combined treatment with TMZ and imipramine further increased the expression of Bax and Caspase-3 compared with cells treated with TMZ and imipramine alone ($P < 0.05$). The expression level of Caspase-3 was also significantly higher in U87MG cells treated with TMZ and imipramine alone compared to the control group ($P < 0.05$; Figure 3). Co-treatment with

Table 1. Primer Sequences Used for qRT-PCR Analysis

Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Product Size (bp)
PI3K	TGAACCTGGACTCTGACAAATG	CTCAGTGATGTCGTCCTCATAG	152
AKT	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT	139
Bax	TGGAGCTGCAGAGGATGATTG	GAAGTTGCCGTCAGAAAACATG	145
Bcl-2	GGTGGGGTCATGTGTGTGG	CGGTTCAGGTACTCAGTCATCC	158
Caspase-3	CATGGAAGCGAATCAATGGACT	CTGTACCAGACCGAGATGTCA	143
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	150

Note. qRT-PCR: Quantitative real-time polymerase chain reaction.

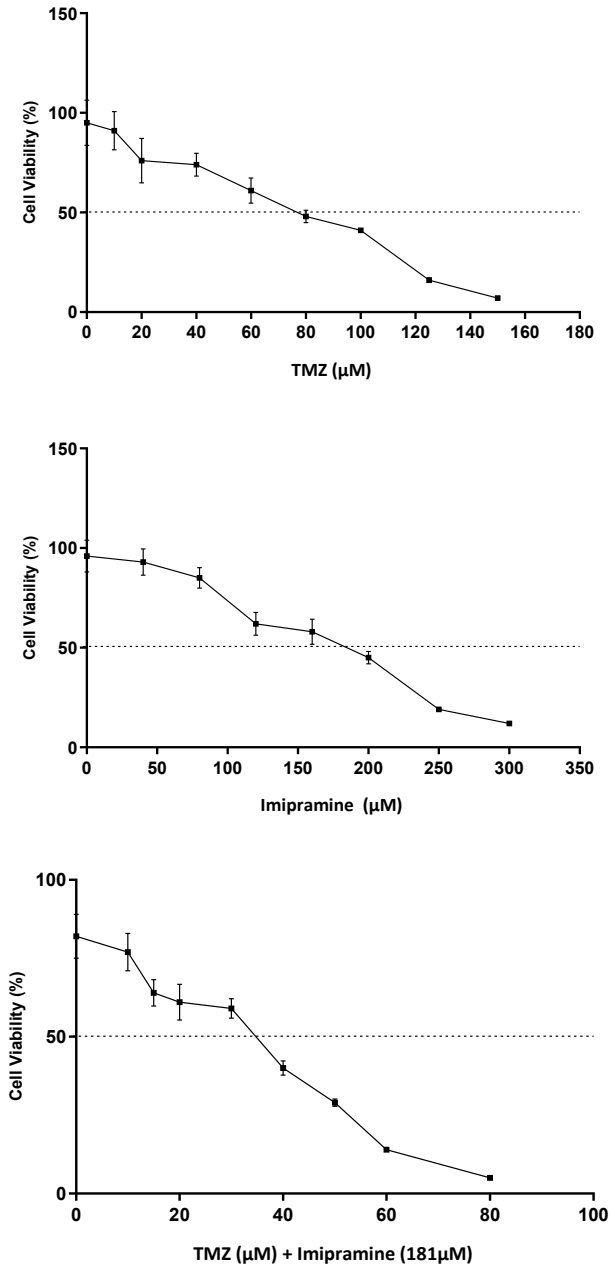

Figure 1. The Effects of TMZ, Imipramine, and Their Combination on U87MG Cell Viability ($P<0.05$) *Note.* Data are presented as mean ± SD of three independent experiments. TMZ: Temozolomide

LY294002, TMZ, and imipramine also caused a greater increase in the expression of both genes ($P<0.05$).

The expression level of the Bcl-2 gene, an anti-apoptotic gene, displayed the opposite pattern. Bcl-2 expression was significantly reduced in U87MG cells treated with TMZ or imipramine alone compared with the control group ($P<0.05$; Figure 3). Combined treatment with TMZ and imipramine caused a greater decrease in Bcl-2 expression ($P<0.05$), and the addition of LY294002 further decreased Bcl-2 expression ($P<0.05$).

Treatment with TMZ or imipramine alone reduced Bax and Caspase-3 expression, whereas their combined treatment led to a significant decrease in U87MG cells. Co-treatment with LY294002, TMZ, and imipramine resulted in an additional reduction in PI3K and AKT expression ($P<0.05$). In contrast, treatment with TMZ

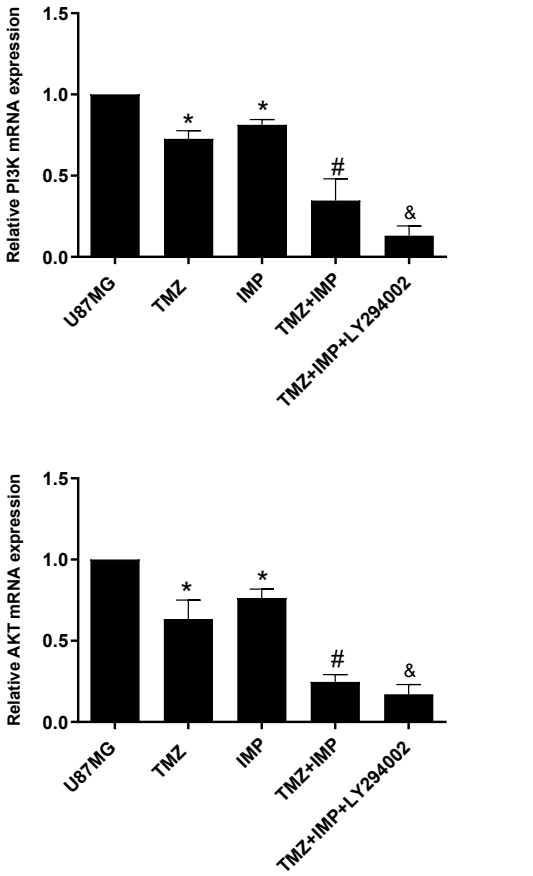

Figure 2. TMZ and Imipramine Modulate PI3K and AKT Expression in U87MG Cells. *Note.* TMZ: Temozolomide

or imipramine alone increased Bcl-2 expression, and the combined treatment and combination with LY294002 led to a notable increase ($P<0.05$). Data represent mean ± SD of three independent experiments.

Inhibition of Imipramine Potentiates Temozolomide-Induced Apoptosis

The activities of Caspase-3 and Caspase-7 enzymes were significantly increased in the combined treatment group compared with the single-treatment groups ($P<0.05$; Figure 4). The caspases activity levels in the combined group were more than twofold higher than in the control group, confirming the induction of apoptotic cell death. Similar results were reported using the ELISA cell-death assay, where combined treatment with TMZ and imipramine led to a significant increase in apoptosis compared with single treatments ($P<0.05$; Figure 4).

Analyses revealed higher caspase-3/caspase-7 activity and a greater apoptotic rate in the combined-treatment group ($P<0.05$). Treatment with TMZ, imipramine, and LY294002 combination caused a higher level of apoptosis ($P<0.05$). Data are presented as mean ± SD of three independent experiments.

Discussion

The results of this study showed that treatment of glioblastoma cells with the standard drug TMZ reduced cell survival and proliferation dose-dependently. However, the relatively high IC50 value indicated the

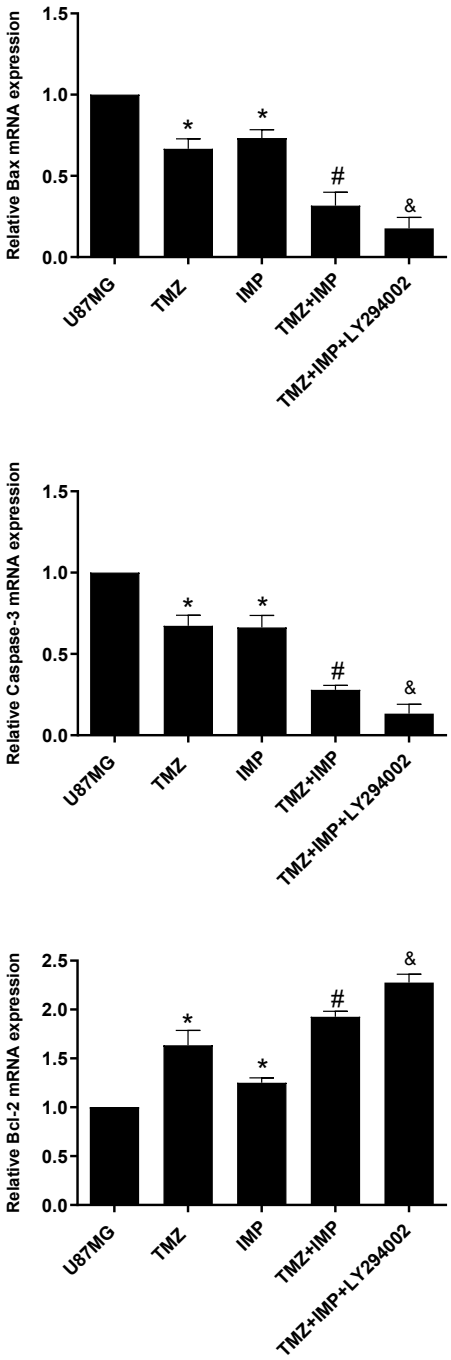

Figure 3. TMZ and Imipramine Modulate Bax, Caspase-3, and Bcl-2 Expression in U87MG Cells. *Note.* TMZ: Temozolomide

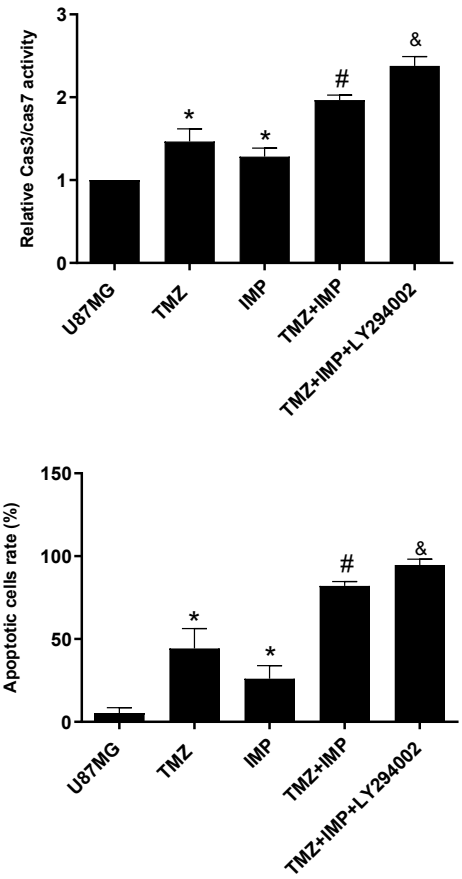

Figure 4. Imipramine Potentiates TMZ-induced Apoptosis. *Note.* TMZ: Temozolomide

intrinsic resistance of the U87MG cell line to TMZ, a resistance repeatedly reported in the scientific literature and is considered one of the main obstacles in glioblastoma treatment. In contrast, imipramine, a tricyclic antidepressant, inhibited cell growth when used alone and showed a dose-dependent cytotoxic effect. This finding is consistent with the report by Jeon et al, who demonstrated that imipramine induces cell death in glioblastoma through inhibition of the PI3K/Akt/mTOR pathway and the induction of autophagy.[15] Similarly, Shchors et al reported that tricyclic antidepressants can activate programmed cell death pathways.[20] This finding suggests that imipramine alone is capable of modulating survival mechanisms in cancer cells, but due to the high intrinsic resistance of GBM cells, monotherapy remains insufficient for effective tumor inhibition. In the present study, combining imipramine with TMZ produced a significant synergistic effect, reducing the IC50 of TMZ from 78 μM to 35 μM. This suggests that imipramine significantly increased cellular sensitivity to TMZ, causing tumor cell death at substantially lower TMZ concentrations. These findings are similar to those of Wang et al, who showed that imipramine combined with TMZ demonstrated significant anti-tumor efficacy in both cellular and animal models.[21] Furthermore, studies by Munson et al on the imipramine derivative called *Imipramine Blue*, which effectively inhibits glioma cell invasion, confirm that derivatives of this drug can enhance the action of standard therapies.[22] Therefore, it can be concluded that the synergistic effect observed in this study is not isolated but rather represents a recurring pattern in different studies, highlighting the potential of imipramine to enhance the efficacy of antitumor drugs.

Investigation of the molecular mechanisms underlying this synergy showed that combined treatment with imipramine and TMZ caused a significant reduction in PI3K and Akt expression compared with each drug alone. The inclusion of LY294002, a specific PI3K inhibitor, served as a critical validation tool for the current study. The observation that LY294002 further enhanced the downregulation of PI3K/Akt and increased apoptotic markers to near-maximal levels provides strong evidence

that the observed synergy is mediated primarily through PI3K/Akt pathway inhibition. This pharmacological validation strengthens the mechanistic interpretation and suggests that achieving optimal therapeutic benefit may require extensive or complete blockade of this survival pathway.

The PI3K/Akt pathway is one of the most central cell survival pathways in glioblastoma and is directly associated with resistance to TMZ. The present finding is consistent with the report by Jeon et al and the systematic review by Lyne et al, both of which highlighted the role of imipramine in inhibiting PI3K/Akt signaling and inducing cell death.[15,23] In addition, Li et al demonstrated that imipramine can also target EGFR receptor and its mutant isoform EGFRvIII, a mechanism that can indirectly lead to a decrease in PI3K/Akt activity.[24] Therefore, the present results clearly demonstrate that the synergy between imipramine and TMZ is achieved through the multifaceted inhibition of survival signaling pathways.

In addition to the effect on the PI3K/Akt pathway, the present study showed that the combination of imipramine and TMZ significantly changed the expression pattern of apoptotic genes. Increased Bax and Caspase-3 expression accompanied by decreased Bcl-2 expression indicates activation of the mitochondrial apoptotic pathway. These gene expression changes suggest that imipramine can facilitate programmed cell death by increasing the Bax/Bcl-2 ratio and promoting caspase activation. The present findings are consistent with the study by Bielecka-Wajdman et al, who showed that tricyclic antidepressants induce cell death through mitochondrial stress and disruption of energy metabolism.[25] Shchors et al also reported that imipramine induced autophagy, confirming that this drug can induce cell death through multiple pathways.[20] Accordingly, it can be argued that the sensitization of GBM cells to TMZ in the presence of imipramine results from the overlap of two mechanisms: inhibition of the PI3K/Akt pathway and activation of the mitochondrial apoptosis pathway.

ELISA-based apoptosis analysis also showed that in the combination treatment group, the percentage of apoptotic cells exceeded 82%, and co-treatment with PI3K inhibitor increased this rate to 94%. These results highlight the pivotal importance of the PI3K/Akt pathway in drug resistance and demonstrate the powerful modulatory role of imipramine in disrupting this pathway. The results are consistent with the findings of Wang et al, who reported a strong synergistic effect between imipramine and TMZ in animal models,[21] and with the review by Petrosyan et al, which identified imipramine as a simultaneous regulator of autophagy and apoptosis.[26] Thus, the present findings not only prove the efficacy of imipramine in enhancing the effect of TMZ but also highlight the position of the PI3K/Akt pathway as a pivotal target for overcoming drug resistance in glioblastoma. Overall comparison of the results of this study with the literature shows that almost all findings are in line with the existing data and have a high convergence with previous studies. The differences are mostly in the magnitude of responses or specific mechanistic details, which can be due to differences in cell lines, culture conditions, drug concentrations, or experimental design. Collectively, the evidence indicates that imipramine acts as a multimodal agent that simultaneously targets several critical tumor pathways, including PI3K/Akt inhibition, EGFR modulation, activation of the mitochondrial pathway, and induction of autophagy. This feature not only enhances TMZ activity but also reduces cancer cell survival and significantly overcomes drug resistance.

From an analytical perspective, these findings indicate that repurposing known drugs such as imipramine could be a cost-effective and safe approach to enhance standard glioblastoma treatments. Given the well-established pharmacokinetic and safety profile of this drug in psychiatric applications, imipramine's use as an adjuvant therapy could pave the way for the development of new clinical protocols. The present results can also provide experimental evidence that simultaneously inhibiting pro-survival signaling pathways and activating cell death pathways is an effective strategy for overcoming the drug resistance barrier in glioblastoma.

While our data strongly implicate the PI3K/Akt pathway as a key mediator of imipramine's sensitizing effects, we acknowledge that this drug likely acts through multiple mechanisms. Previous studies have demonstrated that imipramine can modulate EGFR signaling,[27] induce autophagy,[28] disrupt mitochondrial function,[29] and affect lysosomal pH.[30] The relative contribution of each pathway to the overall therapeutic effect remains to be fully determined and may vary depending on cellular context and genetic background. Our study focused on PI3K/Akt due to its well-established role in TMZ resistance, but comprehensive pathway analyses in future studies will be important to map the complete mechanistic landscape.

A major limitation of the current study is the use of a single glioblastoma cell line (U87MG). While U87MG is a well-established and widely used model, it does not capture the extensive molecular and genetic heterogeneity that characterizes glioblastoma in patients. GBM tumors exhibit remarkable inter- and intra-tumoral heterogeneity, with diverse molecular subtypes (proneural, neural, classical, and mesenchymal) that may respond differently to therapeutic interventions.

Future studies should validate these findings across multiple GBM cell lines with different genetic backgrounds, including models with varying MGMT methylation status, TP53 mutations, and EGFR amplification. Most importantly, validation in patient-derived xenograft models and primary patient-derived cell cultures would provide critical evidence of therapeutic relevance across the spectrum of GBM heterogeneity. Such studies would also enable assessment of whether specific molecular subtypes exhibit greater sensitivity to the imipramine-TMZ combination.

Another limitation of this study is the reliance on gene expression analysis without corresponding protein-

level validation. While qRT-PCR provides valuable information about transcriptional changes, it does not directly confirm alterations in protein abundance or, critically, post-translational modifications such as Akt phosphorylation (p-Akt at Ser473 and Thr308), which serve as functional pathway readouts. Western blot analysis of total and phosphorylated forms of key proteins (PI3K, Akt, mTOR, Bax, Bcl-2, and cleaved caspase-3) would therefore provide more direct evidence of pathway modulation and is planned for follow-up studies.

Furthermore, the absence of in vivo validation represents a significant gap. Although our in vitro findings are promising, they do not account for pharmacokinetic behavior, blood-brain barrier penetration, tumor microenvironment effects, or potential systemic toxicity. Animal studies using orthotopic GBM xenograft models are essential to determine whether the synergistic effects observed in culture translate to tumor growth inhibition and survival benefit in living organisms. Such studies should include assessment of drug distribution to brain tissue, evaluation of optimal dosing schedules, and monitoring of potential adverse effects.

Conclusion

The findings of this study showed that imipramine, as a repurposed drug, can significantly enhance glioblastoma cell sensitivity to TMZ. This effect appears to be substantially mediated through inhibition of the PI3K/Akt signaling pathway and modulation of apoptosis-related genes, characterized by increased Bax and Caspase-3 expression and decreased Bcl-2, as evidenced by the enhanced effects observed with PI3K inhibition. However, imipramine may act through multiple mechanisms, including autophagy induction, EGFR modulation, and mitochondrial stress, which may contribute to the observed synergy. Future studies employing pathway-specific inhibitors and comprehensive signaling analyses will be needed to fully elucidate the relative contribution of each mechanism.

Given the well-established safety profile and favorable pharmacokinetics of imipramine from decades of clinical use in psychiatry, these findings suggest the potential for relatively rapid clinical translation, if validated in appropriate preclinical models. The combination of a known-safe psychiatric medication with standard GBM therapy represents a pragmatic and cost-effective approach to address treatment resistance in this highly aggressive disease. However, rigorous validation, including protein-level analyses, diverse cell-line testing, and animal model studies, is essential before clinical implementation can be considered.

Ethics statement

All experimental procedures were applied following the approval from the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.FMD.REC.1404.122)

Disclosure of funding source

This paper is the outcome of the Medical Degree Thesis (Thesis no. 76611) approved by Tabriz University of Medical Sciences.

Conflict of interests declaration

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to thank Shohada Clinical Research Development Unit, Shohada Hospital, Tabriz University of Medical Science, Tabriz, Iran.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

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Validation: Mohammad Shimia.
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Writing–review & editing: Mohammad Shimia.

Consent for publication

Not applicable.

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