

A novel quinazoline derivative induces apoptosis and modulates EGFR/PI3K/AKT/mTOR-related gene expression in glioblastoma cells

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Abstract

Glioblastoma multiforme (GBM) remains one of the most aggressive and treatment-resistant brain tumors, highlighting the urgent need for novel therapeutic approaches. In this study, we synthesized and characterized a series of quinazoline derivatives and evaluated their antitumor potential in human glioblastoma cells. Among the synthesized compounds, N²-(4-(benzyloxy)phenyl)-6,7-dimethoxyquinazoline-2,4-diamine (KDY23) exhibited the highest antiproliferative activity. Cell viability assays demonstrated significant cytotoxicity in U87-MG and T98G cells, with IC₅₀ values of 6.11 μM and 5.65 μM, respectively. Preliminary selectivity assessment in non-tumoral J774.1 macrophages revealed an IC₅₀ of approximately 12.23 μM, indicating moderate preferential cytotoxicity toward glioblastoma cells. Morphological analyses confirmed substantial reductions in cell density following treatment. Mechanistically, KDY23 induced apoptosis, as evidenced by increased expression of cleaved caspase-3 and cleaved PARP. Furthermore, qRT-PCR analyses revealed marked downregulation of *EGFR*, *PI3K* and *AKT1* transcripts, suggesting modulation of genes associated with the EGFR/PI3K/AKT signaling axis. These findings indicate that KDY23 exerts potent antiglioblastoma activity, displaying moderate tumor selectivity, suppression of EGFR/PI3K/AKT-related gene expression, and activation of apoptotic pathways. Collectively, the results support further preclinical development of KDY23 as a potential therapeutic candidate for glioblastoma.

Highlights

- KDY23 exhibited potent antiproliferative activity against U87-MG and T98G glioblastoma cells.
- KDY23 induced apoptosis through increased cleavage of caspase-3 and PARP.
- Treatment downregulated , , and gene expression.
- KDY23 displayed greater activity than temozolomide under identical experimental conditions.
- Preliminary selectivity assessment demonstrated lower sensitivity of non-tumoral J774.1 macrophages.

1. INTRODUCTION

Glioblastoma multiforme (GBM) is the most common, aggressive, and lethal primary malignant brain tumor in adults. Under standard therapeutic regimens, the prognosis for GBM patients remains dismal, characterized by a five-year survival rate below 10% and a median overall survival of approximately 15 months after diagnosis [1–4]. This clinical failure is largely driven by the tumor's high degree of invasiveness, extreme intratumoral heterogeneity, and intrinsic or acquired therapeutic resistance, which collectively highlight the urgent need for novel molecularly targeted approaches to reduce disease-associated morbidity and mortality [1–3].

Current clinical guidelines recommend a multidisciplinary approach as the standard of care for newly diagnosed patients, comprising maximal safe surgical resection followed by radiotherapy combined with concurrent and adjuvant chemotherapy using temozolomide (TMZ) [5–6]. TMZ, an oral alkylating agent capable of crossing the blood-brain barrier, remains the cornerstone of systemic therapy for both newly diagnosed and recurrent GBM [2, 6]. Although widely utilized, TMZ frequently causes severe systemic adverse effects during prolonged administration, and its long-term efficacy is severely limited by the almost universal development of chemoresistance [7, 8]. At the cellular level, resistance to TMZ is dynamically coordinated by enhanced DNA repair capacity, O⁶-methylguanine-DNA methyltransferase (MGMT) overexpression, and the compensatory activation of pro-survival signaling networks [7–9]. Consequently, identifying novel small molecules capable of overcoming TMZ resistance mechanisms has become a major priority in neuro-oncology research [2, 3].

Among the core driving mechanisms of GBM pathogenesis and chemoresistance, receptor tyrosine kinases—particularly the epidermal growth factor receptor (EGFR)—play a central role in sustaining tumor growth and therapeutic tolerance [10–13]. The complex molecular landscape of GBM is frequently characterized by *EGFR* amplification, *PTEN* loss, and *TP53* mutations, which culminate in the constitutive hyperactivation of downstream survival signaling cascades [12, 14, 15]. Chief among these is the phosphoinositide 3-kinase/Akt/mammalian target of rapamycin PI3K/AKT/mTOR pathway, an attractive therapeutic target that critically regulates metabolic adaptation, uncontrolled proliferation, and apoptotic evasion [3, 12]. Therefore, targeted therapeutic strategies designed to dismantle this specific oncogenic axis, while simultaneously bypassing or reversing conventional chemotherapy resistance, have emerged as highly promising frontiers in glioblastoma management [3, 12, 14, 16].

Quinazoline derivatives constitute one of the most successful classes of kinase inhibitors in oncology. Several clinically approved anticancer agents, including gefitinib, erlotinib, afatinib and lapatinib, are based on the quinazoline scaffold and exert their activity through inhibition of receptor tyrosine kinases, particularly EGFR [17–19]. Due to their favorable pharmacological properties and ability to modulate signaling pathways involved in tumor progression, quinazoline derivatives have attracted considerable attention as potential therapeutic agents for glioblastoma and other solid tumors [18–20].

Furthermore, the induction of apoptosis is considered one of the most desirable characteristics of anticancer agents. Activation of caspase-3 and PARP cleavage are hallmarks of apoptotic cell death and are frequently associated with the antitumor activity of successful chemotherapeutic drugs [21, 22]. Therefore, compounds capable of simultaneously suppressing oncogenic signaling pathways and activating apoptotic mechanisms may represent promising candidates for glioblastoma treatment.

In this context, the present study investigates the antitumor potential of KDY23, a novel quinazoline-based compound with structural features commonly associated with kinase inhibition. The findings presented herein identify KDY23 as a promising lead compound capable of reducing glioblastoma cell viability, inducing apoptosis and modulating the expression of genes associated with the EGFR/PI3K/AKT signaling axis.

2. MATERIALS AND METHODS

2.1. Synthesis of quinazoline derivatives

General procedure for the synthesis of quinazoline derivatives: The quinazoline derivatives were synthesized, purified and characterized following methodologies adapted from our current patent registry [Patent BR 10 2024 019192 7] and literature standards for quinazoline scaffolds [17, 19]. In a 50 mL round-bottom flask, 5.08 mmol of the 2-chloro-6,7-dimethoxy-4-quinazolinamine, 45.4 mmol (9 equivalent) of the aniline derivative and 30 mL of isopropanol were added. The reaction mixture was refluxed under magnetic stirring for 12 h. Next, the product was vacuum filtered using qualitative filter paper and washed abundantly with isopropanol. The thin-layer chromatography (TLC) analysis revealed the presence of residual precursor (1) in the product samples. (methanol/ethyl acetate 1:9 v/v). To address this, 25 mg of the solid was weighed into 15 mL Falcon tubes, to which 15 mL of distilled water were added. The samples were vortexed until complete dispersion, followed by centrifugation at 3000 rpm for 2 minutes, resulting in the formation of a white pellet at the bottom of the tubes. The supernatant was carefully removed using a Pasteur pipette. This washing procedure was repeated once more under identical conditions. After freeze drying, the pure product was isolated.

2.2. Structural characterization of KDY23

The chemical structure and purity of N²-(4-(benzyloxy)phenyl)-6,7-dimethoxyquinazoline-2,4-diamine (KDY23) were determined using melting point determination, Fourier-transform infrared spectroscopy (FT-IR), and nuclear magnetic resonance (¹H and ¹³C NMR). The obtained spectroscopic data were indexed as follows: white solid (78% yield); mp 260–265°C; IR (KBr, cm⁻¹): 3426, 3303, 3286, 3204, 3171, 3118, 3036, 3001, 2929, 2874, 2851, 1655, 1629, 1608, 1508, 1489, 1438, 1399, 1231, 1214, 1110, 1005, 829, 753, 702, 525;

¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm) 12.10 (br, 1H), 10.04 (s, 1H), 8.95 (s, 1H), 8.73 (s, 1H), 7.76 (s, 1H), 7.48–7.39 (m, 6H), 7.34 (t, *J* = 7.2 Hz, 1H), 7.09–7.07 (m, 2H), 5.13 (s, 2H), 3.88 (s, 3H), 3.84 (s, 3H);

¹³C {¹H} NMR (125 MHz, DMSO-*d*₆) δ (ppm) 162.0, 156.2, 155.6, 151.6, 146.9, 137.0, 135.5, 129.5, 128.5, 127.9, 127.7, 125.4, 115.4, 105.1, 102.2, 98.8, 69.5, 56.3, 56.2.

2.3. Cell culture

The U87-MG and T98G cell lines were used as in vitro models of glioblastoma multiforme. Although U87-MG remains one of the most widely used glioblastoma models, future studies employing patient-derived glioblastoma cells would strengthen translational relevance. U87-MG cells were kindly provided by CIPOL (Integrated Center for Onco-Hematological Research in Childhood, UNICAMP), while T98G cells were obtained from the Rio de Janeiro Cell Bank (BCRJ, code 0406). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

2.4. Cell viability assay (MTT)

Cell viability was determined using the MTT assay based on the reduction of tetrazolium salt by mitochondrial dehydrogenases in viable cells.

For the assays, cells were seeded in 96-well plates at a density of 2×10^5 cells/mL and allowed to adhere for 24 h. Subsequently, cells were exposed to KDY23 at concentrations of 1, 5, 7.5, 10, 25 and 50 μM for 24 h. Temozolomide (400 μM) was used as reference drug. DMSO was used as solvent and PBS-treated cells served as controls. The final concentration of DMSO did not exceed 0.5%. Cell viability was expressed as percentage relative to the control group and IC_{50} values were calculated by nonlinear regression analysis.

2.5. Evaluation of cytotoxicity in non-tumoral cells

To obtain preliminary information regarding tumor selectivity, murine macrophage J774.1 cells were exposed to increasing concentrations of KDY23 under the same experimental conditions employed for glioblastoma cells.

2.6. Western blot analysis

T98G cells were lysed in RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) containing protease and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA, USA). After centrifugation, protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked with bovine serum albumin (BSA; Sigma-Aldrich) and incubated overnight at 4°C with primary antibodies against rabbit cleaved caspase-3 (1:1000; Cell Signaling Technology, Danvers, MA, USA), rabbit cleaved PARP (1:1000; Cell Signaling Technology) and mouse β -actin (1:5000; Sigma-Aldrich) as the endogenous loading control. After washing three times with TBS-T, the membranes were incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-rabbit IgG or anti-mouse IgG, 1:3000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Bio-Rad Laboratories, Hercules, CA, USA) and quantified by densitometric analysis using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.7. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from treated and untreated T98G cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in strict accordance with the manufacturer's protocol. The RNA concentration and purity were assessed spectrophotometrically using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), tracking A_{260}/A_{280} ratio. Complementary DNA (cDNA) synthesis was performed using 1 μg of total RNA reverse-transcribed with the High-Capacity cDNA Reverse

Transcription Kit (Applied Biosystems, Foster City, CA, USA) in a final volume of 20 μ L. Quantitative real-time PCR (qRT-PCR) assays were executed using a 7500 Fast Real-Time PCR System (Applied Biosystems) utilizing the PowerTrack SYBR Green Master Mix (Applied Biosystems). Each amplification reaction contained 2 $\times$ SYBR Green Master Mix, 300 nM of each forward and reverse target-specific primer, 2 μ L of diluted cDNA template, and nuclease-free water to a final volume of 10 μ L. The thermal cycling conditions consisted of an initial activation step at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 5 s and annealing/extension at 60°C for 30 s. Melt-curve analysis (60°C to 95°C) was performed immediately after amplification to verify single-product specificity. The specific primer sequences for human genes included *EGFR*, *AKT1*, *PIK3CA*, *MTOR*, *MAPK1*, *MAPK3*, *NFKB1*, and *NFKB2*, with *GAPDH* serving as the stable internal reference gene (Table 1). Relative mRNA expression levels were calculated using the comparative $2^{-\Delta\Delta C_t}$ method.

Table 1 Primer sequences, amplicon sizes, and amplification efficiencies utilized for qRT-PCR gene expression analysis in human glioblastoma cells.

Gene	Forward Primer Sequence (5' $\longrightarrow$ 3')	Reverse Primer Sequence (5' $\longrightarrow$ 3')	Amplicon Size	Efficiency
<i>EGFR</i>	TGGCACCCAGCACAATGAAG	ACTGTGTTGGCGCACCCTA	110 bp	98%
<i>PIK3CA</i>	CCACGACCATCATCAGGTGAA	CCTCACGGAGGCATTCTAAAGT	134 bp	99%
<i>AKT1</i>	AGCGACGTGGCTATTGTGAAG	GCCATCATTCTTGAGGAGGAAGT	142 bp	97%
<i>MTOR</i>	ATGCTTGGAACCGGACCTTG	TTTCTCAGACGTTTCCGCTC	125 bp	96%
<i>MAPK1</i>	CCGCTGACCCCGATGAGC	ACCTGTGATCTCCATCCTCA	118 bp	98%
<i>MAPK3</i>	TCCGCCATGAGAATGTTATA	ACAGTTAGGTTTGTCGTCGT	105 bp	95%
<i>NFKB1</i>	AACACTGTCCTGCCTCTATC	CTAGATAGAGGCAGGACAGT	150 bp	99%
<i>NFKB2</i>	ATCTCATTGAGGTGGCTCGG	GTCAGAGCCCCGTAGAAGTT	138 bp	97%
<i>GAPDH</i>	CCTCTGACTTCAACAGCGAC	TCTCTCTTCTCTTGTGCTC	121 bp	100%

2.8. In silico prediction of blood-brain barrier permeability

The blood-brain barrier permeability of KDY23 was predicted using the ADMETlab 3.0 platform. The molecular structure of KDY23 was submitted to the platform and the probability of BBB penetration was calculated according to the software algorithm.

2.9. Statistical analysis

All experiments were performed independently at least three times and each condition was evaluated in triplicate. Data are presented as mean $\pm$ SEM. Statistical analyses were performed using GraphPad Prism software. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Differences were considered statistically significant when $p < 0.05$.

3. RESULTS

3.1 Chemical Synthesis and Initial Screening

The quinazoline derivative N²-4-(benzyloxy)phenyl-6,7-dimethoxyquinazoline-2,4-diamine (KDY23) was successfully synthesized with an isolated yield of 78%. Structural characterization confirmed its high purity and successfully mapped the expected functional groups and framework architecture. In the IR spectrum, the presence of primary and secondary amine groups was confirmed by characteristic N–H stretching bands between 3426 and 3118 cm⁻¹, while typical ether C–O stretchings appeared at 1231 and 1214 cm⁻¹.

The ¹H NMR spectrum provided definitive evidence for the structure through a sharp singlet at δ 5.13 ppm (2H), corresponding to the benzylic methylene protons (–CH₂–), along with two intense singlets at δ 3.88 and 3.84 ppm (3H each) that validated the preservation of the two methoxy groups (–OCH₃–) on the quinazoline core.

The target skeleton was further corroborated by ¹³C NMR spectroscopy, which resolved the characteristic heterocyclic carbon resonances downfield (δ 162.0–146.9 ppm) and successfully identified the aliphatic sp³ carbons corresponding to the benzylic (δ 69.5 ppm) and symmetric methoxy positions (δ 56.3 and 56.2 ppm). Collectively, these data confirm that the modifications to the privileged quinazoline scaffold were precisely accomplished.

Quinazoline derivatives represent a highly privileged scaffold in oncology, serving as the structural basis for several FDA-approved tyrosine kinase inhibitors like gefitinib and erlotinib, which target EGFR. Our initial screening at 10 μ M supported the selection of KDY23 for further biological investigation (Fig. 1).

3.2. Antiproliferative Activity and Tumor Selectivity

KDY23 induced a concentration-dependent reduction in cell viability across both glioblastoma cell lines, yielding IC₅₀ values of 6.11 μ M for U87-MG and 5.65 μ M for T98G. As outlined in the introduction, temozolomide (TMZ) is the cornerstone of GBM chemotherapy, but its efficacy is severely hindered by resistance mechanisms. Literature reviews show that the average IC₅₀ of TMZ is 240.0 μ M for U87-MG and 438.3 μ M for the resistant T98G line. In contrast, KDY23 exhibited drastically lower IC₅₀ values under identical conditions (Fig. 2). Notably, T98G cells naturally overexpress DNA repair genes and MGMT, making them highly resistant to alkylating agents; however, KDY23 maintained antiproliferative activity in

the TMZ-resistant T98G cell line, reducing T98G viability starting at 5 μ M (31.81% reduction). Morphological assessment via methylene blue staining visually sustained these findings, revealing a severe drop in cell density and prominent cytological alterations (Fig. 3). To establish a preliminary therapeutic window, toxicity was tested on non-tumoral J774.1 macrophages. KDY23 exhibited an IC_{50} of ~ 12.23 μ M, resulting in a selectivity index of 1.6 to 1.8, suggesting a modest preferential cytotoxic profile toward malignant cells (Fig. 4).

3.3. Activation of Apoptotic Pathways

Evading programmed cell death is a fundamental hallmark of glioblastoma progression. To determine whether the antiproliferative effects of KDY23 were linked to apoptosis, the cleavage of caspase-3 and PARP was evaluated. Densitometric quantification normalized to β -actin revealed a 3.5-fold increase in cleaved caspase-3 and a remarkable 5-fold increase in cleaved PARP levels following KDY23 treatment (10 μ M) compared to the vehicle control (Fig. 5). Because caspase-3 activation and subsequent PARP degradation represent the definitive execution phase of apoptosis, these molecular signatures suggest that KDY23 induces activation of apoptotic pathways in treatment-resistant T98G cells.

3.4. Transcriptional Modulation of EGFR/PI3K/AKT-Related Genes

Aberrant activation of receptor tyrosine kinases, particularly through EGFR amplification and constitutive downstream PI3K/AKT/mTOR signaling, drives cell survival, proliferation, and therapeutic resistance in 50–60% of GBM cases. Our qRT-PCR analyses revealed that KDY23 induced a coordinated downregulation of this oncogenic axis. KDY23 suppressed *EGFR* transcript levels by $\sim 71\%$ (compared to a modest 39% by TMZ) and decreased *PIK3CA* and *AKT-1* transcripts by $\sim 30\%$ and $\sim 68\%$, respectively. These transcriptional changes suggest that modulation of genes associated with the EGFR/PI3K/AKT pathway may contribute to the antiproliferative and pro-apoptotic effects observed after KDY23 treatment. Interestingly, an upregulation of *mTOR* mRNA was detected after both KDY23 and TMZ treatments. This finding may reflect an adaptive cellular feedback mechanism trying to restore metabolic homeostasis when upstream AKT signaling is inhibited. Nonetheless, this compensatory response was insufficient to rescue the cells from KDY23-mediated apoptosis. No significant alterations were found in *MAPK1* and *MAPK3* expressions, suggesting that the RAS/RAF/MEK/ERK pathway is not the primary target of this quinazoline derivative (Fig. 6).

3.5. In Silico Blood-Brain Barrier (BBB) Permeability

A major bottleneck in neuro-oncology drug development is ensuring that small molecules cross the blood-brain barrier (BBB). Computational modeling via ADMETlab 3.0 predicted a 74% probability of BBB penetration for KDY23. This predicted physicochemical profile suggests potential suitability for central nervous system drug development, supporting future in vivo studies to evaluate brain penetration and pharmacokinetic properties.

4. DISCUSSION

Glioblastoma multiforme (GBM) remains characterized by extensive therapeutic resistance and uniform recurrence, partly driven by intratumoral heterogeneity and rapid proliferation [1–3]. The development of successful therapeutic candidates relies heavily on molecules capable of bypassing traditional chemoresistance while targeting specific oncogenic survival axes [2, 3]. In this study, the synthesis of KDY23 yielded a novel quinazoline derivative that addresses these challenges through its low micromolar efficacy against human glioblastoma models. The quinazoline core is a highly successful chemical scaffold in clinical oncology, serving as the base for several FDA-approved tyrosine kinase inhibitors, such as gefitinib and erlotinib, which competitively target the ATP-binding pocket of receptor tyrosine kinases [17–19]. The superior antiproliferative activity of KDY23 over its structural analogs during initial screenings supports the hypothesis that structural modifications of the quinazoline scaffold may enhance cytotoxic activity against glioblastoma cells. [18–20].

The potent cytotoxicity of KDY23 is highlighted by its low micromolar IC_{50} values against both U87-MG (6.11 μ M) and T98G (5.65 μ M) cells. These outcomes stand in contrast to the historical efficacy profile of temozolomide (TMZ). Meta-analyses of glioma cell line sensitivities demonstrate that the average concentration of TMZ required to achieve an IC_{50} is 240.0 μ M for U87-MG and reaches up to 438.3 μ M for T98G cells [23]. The high tolerance of T98G cells to alkylating agents like TMZ is mediated by enhanced DNA repair capacities, including high levels of *O*⁶methylguanine-DNA methyltransferase (MGMT) and the overexpression of repair genes like *ATM* and *BRCA2* [7–9, 24]. The capacity of KDY23 to decrease viability in T98G cells suggests activity in a model characterized by resistance to alkylating agents. [13]. This is further supported by cytomorphological data from methylene blue staining [25], where KDY23 reduced cell density more effectively than TMZ (400 μ M) or doxorubicin (10 μ M) [21, 22].

Preliminary safety assessments using non-tumoral J774.1 macrophages revealed an IC_{50} of 12.23 μ M. While the calculated selectivity index (1.6 to 1.8) is modest, it points to a preferential cytotoxic action toward glioblastoma lines. However, additional studies using non-tumoral human neural cells are necessary to better define compound selectivity.

Mechanistically, the reduction in cell viability induced by KDY23 is driven by the activation of apoptosis. The evasion of programmed cell death is a critical hallmark of cancer that allows glioblastoma cells to survive genotoxic stress [8, 21]. The robust increase in cleaved caspase-3 (3.5-fold) and cleaved PARP (5-fold) following KDY23 treatment confirms the engagement of the apoptotic cascade. Activation of caspase-3 is a central event in apoptotic signaling, leading to the downstream cleavage of PARP, a nuclear enzyme involved in DNA repair [21, 22]. This mechanism aligns with the actions of established antineoplastic agents, validating KDY23's pro-apoptotic profile [22].

To link these phenotypic outcomes to specific intracellular survival pathways, transcriptional changes were monitored. Aberrant activation of the receptor tyrosine kinase EGFR, often driven by gene amplification, occurs in 50–60% of clinical glioblastoma cases and stimulates tumor growth, invasion,

and chemotherapy resistance [10–13]. Downstream of EGFR, the PI3K/AKT cascade serves as a key survival axis, regulating proliferation and suppressing apoptosis [12, 16]. The coordinated downregulation of *EGFR* (~ 71%), *PI3K* (~ 30%), and *AKT1* (~ 68%) transcripts following KDY23 treatment suggests modulation of genes associated with this signaling cascade [12, 15]. These transcriptional alterations suggest that suppression of genes associated with this signaling axis may contribute to reduced cell survival and activation of caspase-dependent apoptosis [6, 21].

Interestingly, a compensatory increase in *mTOR* transcripts was detected after treatment with both KDY23 and TMZ. This phenomenon likely represents an adaptive cellular feedback loop trying to preserve metabolic homeostasis and survive upstream PI3K/AKT inhibition [16, 26]. Cross-talk with alternative energy sensors frequently induces *mTOR* upregulation during AKT blockade [10]. Crucially, this adaptive response was insufficient to rescue the cells from KDY23-mediated death, as evidenced by the high apoptosis rates. The lack of significant alterations in *MAPK1* and *MAPK3* transcripts indicates that the RAS/RAF/MEK/ERK axis is not a primary target of KDY23, suggesting pathway selectivity.

Resisting cell entry is a major challenge for glioblastoma therapeutics, ensuring adequate blood-brain barrier (BBB) penetration [2, 12]. Small molecules often fail in neuro-oncology because they cannot cross this barrier [12]. The *in silico* prediction assigned a 74% probability of BBB penetration to KDY23. This predicted physicochemical profile supports future *in vivo* pharmacokinetic evaluations to confirm its brain distribution and therapeutic window [2].

4.1. Study limitations

Although KDY23 demonstrated significant antiglioblastoma activity and moderate selectivity in non-tumoral J774.1 macrophages, additional studies using human non-tumoral neural cells are necessary to better characterize its therapeutic window. Furthermore, evaluation of protein phosphorylation within the EGFR/PI3K/AKT pathway would provide additional mechanistic insight. Additionally, the mechanistic interpretation of pathway modulation is limited by the absence of protein-level analyses of EGFR, AKT and mTOR activation status. Future studies evaluating phosphorylation markers will be important to confirm pathway inhibition. Finally, *in vivo* studies are required to assess blood-brain barrier penetration, toxicity and antitumor efficacy before clinical translation can be considered.

CONCLUSIONS

KDY23 exhibited potent antiglioblastoma activity in U87-MG and T98G cells, induced apoptosis through activation of caspase-3 and PARP cleavage, and modulated the expression of genes associated with the EGFR/PI3K/AKT pathway. The compound also demonstrated moderate selectivity toward tumor cells when compared with non-tumoral J774.1 macrophages. Collectively, these findings identify KDY23 as a promising quinazoline-based lead compound for further preclinical investigation in glioblastoma.

Declarations

Author contributions

- **Mariany Lopes da Costa Folly:** Investigation, Formal analysis, Writing – original draft.
- **Antonio Frederico de Freitas Gomides:** Biological assays, Investigation.
- **Jeferson Gomes da Silva:** Methodology, Structural characterization.
- **Pedro Pôssa de Castro:** Chemical synthesis, Data analysis.
- **Ana Carolina Menezes Mendonça Valente:** Investigation.
- **Jullyana Bicalho Costa:** Investigation.
- **Larissa Moura de Matos Franco:** Methodology.
- **Michelle Bueno de Moura Pereira:** Supervision, Funding acquisition, Writing – review & editing.
- **João Eustáquio Antunes:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Data availability

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

Ethical approval

Not applicable.

Consent for publication

Not applicable.

References

1. Chen L, Zou X, Wang Y, Mao Y, Zhou L. Central nervous system tumors: a single center pathology review of 34,140 cases over 60 years. BMC Clin Pathol. 2013;13:14.

2. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: State of the art and future directions. *CA Cancer J Clin.* 2020;70:299–312.
3. Thakur A, Faujdar C, Sharma R, Sharma S, Malik B, Nepali K, et al. Glioblastoma: Current Status, Emerging Targets, and Recent Advances. *J Med Chem.* 2022;65:8596–8685.
4. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS Statistical Report. *Neuro Oncol.* 2019;21:v1–v100.
5. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO Classification of Tumors of the Central Nervous System. *Neuro Oncol.* 2021;23:1231–1251.
6. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352:987–996.
7. Lee SY. Temozolomide resistance in glioblastoma multiforme. *Genes Dis.* 2016;3:198–210.
8. Binabaj MM, Bahrami A, ShahidSales S, Joodi M, Mashhad MJ, Hassanian SM, Avan A. The prognostic value of MGMT promoter methylation in glioblastoma: a meta-analysis. *J Cell Physiol.* 2018;233:378–386.
9. Bobola MS, Kolstoe DD, Blank A, Chamberlain MC, Silber JR. Repair of 3-methyladenine and abasic sites by base excision repair mediates glioblastoma resistance to temozolomide. *Front Oncol.* 2012;2:176.
10. Eskilsson E, Røslund GV, Solecki G, Wang Q, Harter PN, Graziani G, et al. EGFR heterogeneity and implications for therapeutic intervention in glioblastoma. *Neuro Oncol.* 2018;20:743–752.
11. Xu H, Zong H, Ma C, Ming X, Shang M, Li K, et al. Epidermal growth factor receptor in glioblastoma. *Oncol Lett.* 2017;14:512–516.
12. Khabibov M, Garifullin A, Boumber Y, Khaddour K, Fernandez M, Khamitov F, et al. Signaling pathways and therapeutic approaches in glioblastoma multiforme. *Int J Oncol.* 2022;60:69.
13. Sigismund S, Avanzato D, Lanzetti L. Emerging functions of the EGFR in cancer. *Mol Oncol.* 2018;12:3–20.
14. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* 2022;12:31–46.
15. Li XP, Guo ZQ, Wang BF, Zhao M. EGFR alterations in glioblastoma play a role in antitumor immunity regulation. *Front Oncol.* 2023;13:1236246.
16. Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. *Cell Biosci.* 2020;10:31.
17. Al-Rashood ST. Quinazoline derivatives as privileged scaffolds in anticancer drug discovery: recent advances and future directions. *Molecules.* 2024;29:415.
18. Gomaa HAM. A comprehensive review of recent advances in the biological activities of quinazolines. *Chem Biol Drug Des.* 2022;100:639–655.
19. El-Azab AS, Eltahir KEH. Design and synthesis of novel 7-aminoquinazoline derivatives: antitumor activities. *Bioorg Med Chem Lett.* 2012;22:1879–1885.

20. Wdowiak P, Matysiak J, Kuszta P, Czarnek K, Niezabitowska E, Baj T. Quinazoline derivatives as potential therapeutic agents in urinary bladder cancer therapy. *Front Chem.* 2021;9:765552.
21. Mizutani H, Tada-Oikawa S, Hiraku Y, Kojima M, Kawanishi S. Mechanism of apoptosis induced by doxorubicin through the generation of hydrogen peroxide. *Life Sci.* 2005;76:1439–1453.
22. Brenes O, Arce F, Gätjens-Boniche O, Díaz C. Characterization of cell death events induced by anti-neoplastic drugs. *Biomed Pharmacother.* 2007;61:347–355.
23. Poon M, et al. Sensitivity of malignant glioma cell lines to temozolomide: a systematic review and meta-analysis. *BMC Cancer.* 2021;21:541.
24. Pinevich AA, et al. High-dose temozolomide evaluation and MGMT repair activity in resistant glioma models. *Neurochem Res.* 2019;44:1102–1111.
25. Sgouras DN, et al. Methylene blue staining methods for cytomorphological density evaluation. *J Clin Pathol.* 2015;68:311–317.
26. O'Reilly KE, et al. mTOR inhibition induces upstream receptor tyrosine kinase activation and feedback loops in cancer cells. *Cancer Res.* 2006;66: 1500–1508.

Figures

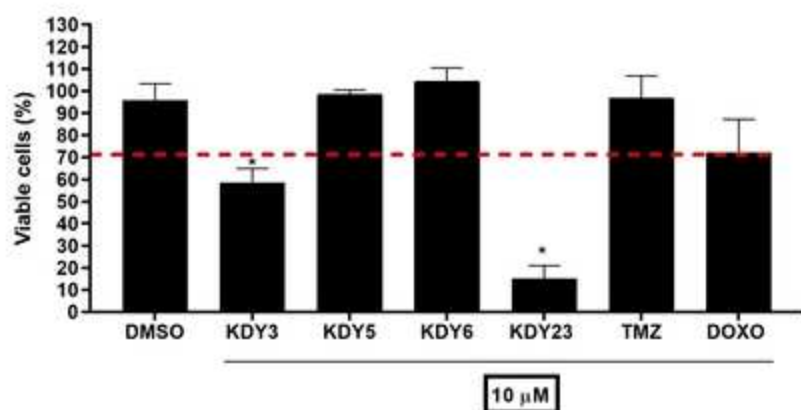


Figure 1

Initial biological screening of synthesized quinazoline derivatives. Antiproliferative activity was evaluated at a fixed concentration of 10 µM against human glioblastoma cell lines after 24 h of exposure. Data are expressed as mean ± standard error of the mean (SEM) of three independent experiments performed in triplicate. Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test (*P < 0.05 versus DMSO control group).

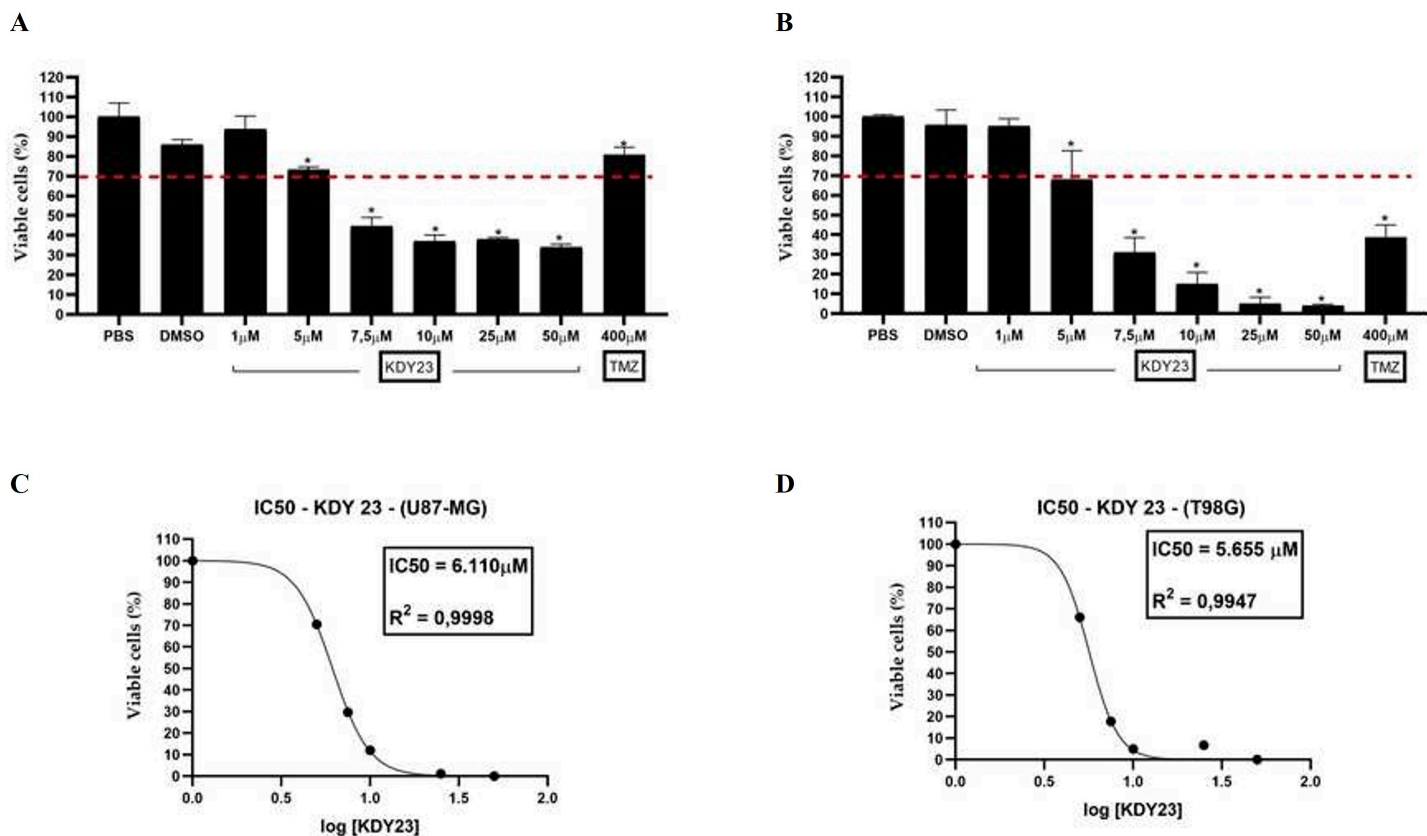


Figure 2

Concentration-dependent antiproliferative profiles and non-linear regression curves of the novel quinazoline derivative KDY23 after 24 h of exposure. **(A)** Concentration-dependent effect of the novel quinazoline derivative KDY23 on the U87-MG glioblastoma cell line. **(B)** Concentration-dependent effect of the novel quinazoline derivative KDY23 on the T98G glioblastoma cell line. **(C)** Non-linear regression and sigmoidal dose-response curve for the determination of the half-maximal inhibitory concentration IC₅₀ of KDY23 in the U87-MG human glioblastoma cell line. **(D)** Non-linear regression and sigmoidal dose-response curve for the determination of the half-maximal inhibitory concentration IC₅₀ of KDY23 in the T98G human glioblastoma cell line. Data represent the mean $\pm$ SEM of three independent biological experiments/replicates executed in triplicate. Statistically significant differences for panels A and B were evaluated via one-way ANOVA followed by Bonferroni's post hoc test (*P < 0.05 versus vehicle control group DMSO).

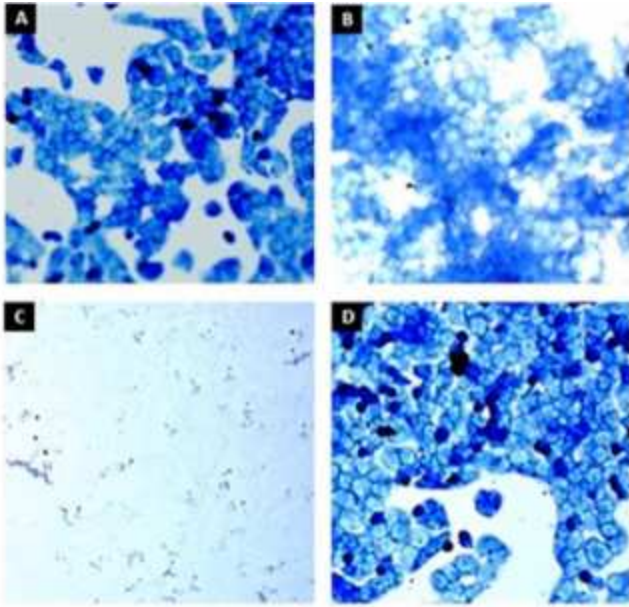


Figure 3

Cytomorphological alterations in human glioblastoma cells. Representative light microscopy images of T98G cells after 24 h of treatment, stained with 0.5% methylene blue. (A) Temozolomide (TMZ) at 400 μ M; (B) doxorubicin at 10 μ M; (C) KDY23 at 10 μ M; (D) vehicle control group (DMSO). Images reflect characteristic cell density reductions and structural modifications from three independent experimental replicates.

IC₅₀ KDY23 (J774.1)

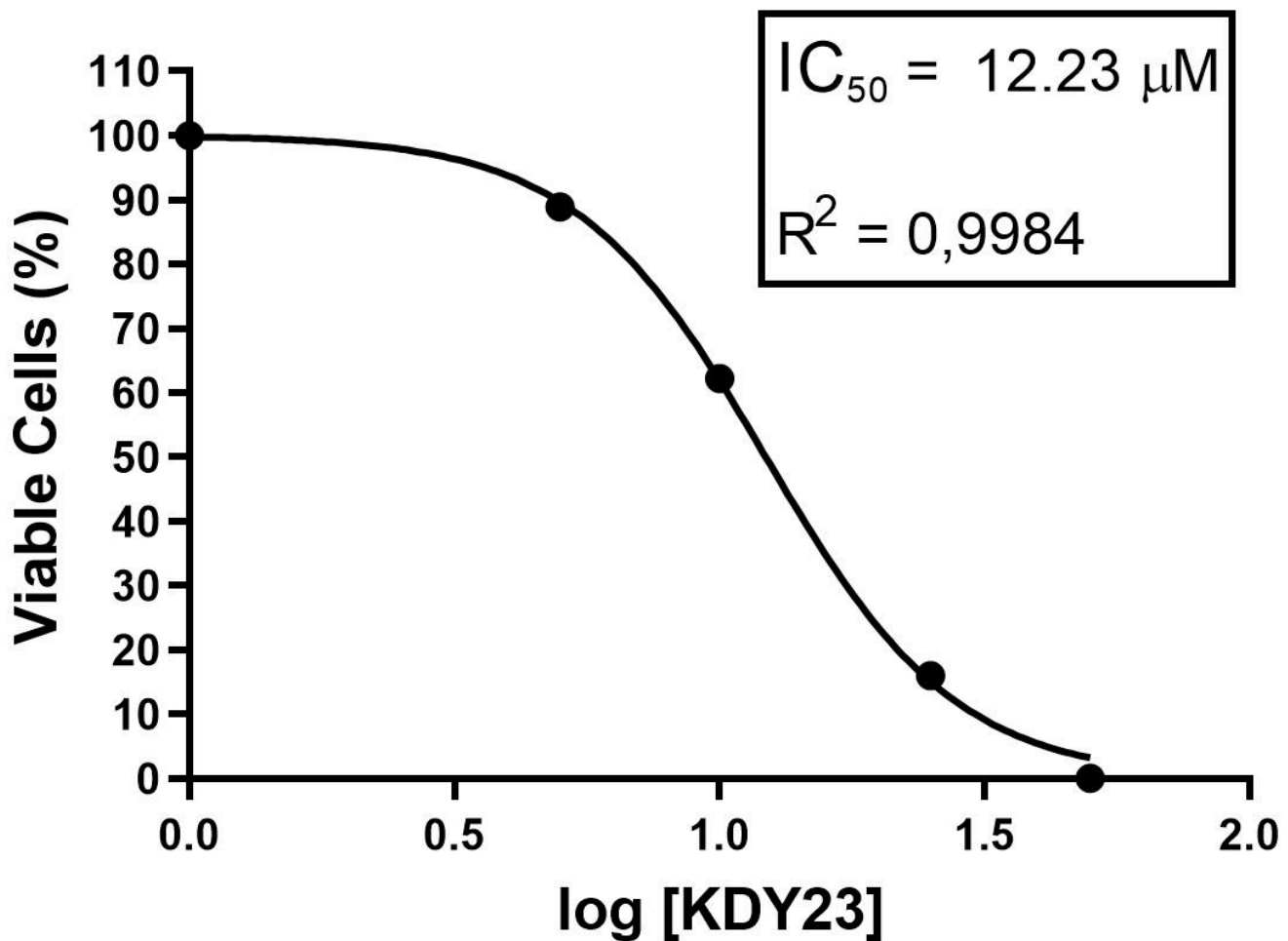


Figure 4

Non-linear regression and sigmoidal dose-response curve for the determination of the half-maximal inhibitory concentration IC₅₀ of KDY23 in the non-tumoral J774.1 macrophages cell line after 24 h of treatment. Data are presented as mean ± SEM of three independent biological replicates.

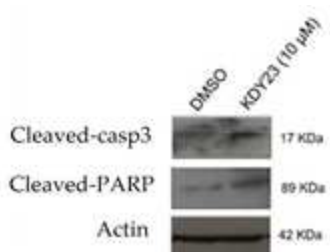


Figure 5

KDY23 triggers caspase-dependent apoptotic pathway executioners in human glioblastoma cells. (A) Representative western blot immunoreactive bands from six independent experiments showing protein extracts from T98G cells treated with vehicle control (DMSO) or KDY23 (10 μ M) for 24 h. Actin was utilized as the stable endogenous loading control. Data represent the mean $\pm$ SEM (n = 6). Significant differences were evaluated via one-way ANOVA with Bonferroni's correction (P < 0.05 compared to the control group).

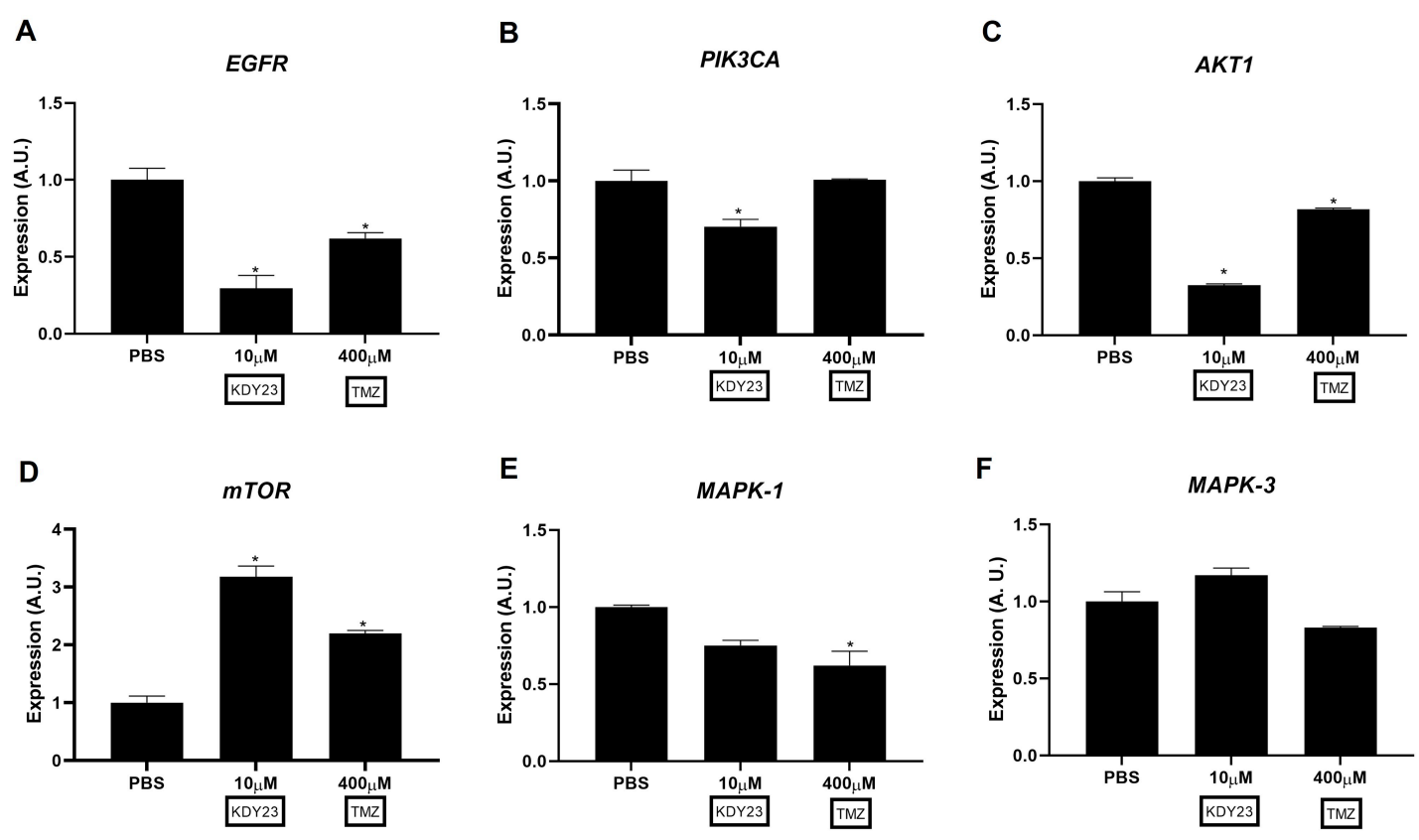


Figure 6

Transcriptional modulation of oncogenic and survival signaling cascades by KDY23. Quantitative real-time PCR (qRT-PCR) evaluation of *EGFR*, *PIK3CA*, *AKT1*, *MTOR*, *MAPK1* and *MAPK3* mRNA expression levels in T98G glioblastoma cells following 24 h of exposure to KDY23 (10 μ M) or TMZ (400 μ M). *GAPDH* was employed as the stable endogenous reference gene for normalization. Relative expression levels ($2^{-\Delta\Delta C_t}$) are expressed as mean $\pm$ SEM of three independent biological replicates (n = 3). Statistically significant differences were evaluated via one-way ANOVA followed by Bonferroni's multiple comparisons test (*P < 0.05 versus vehicle control group DMSO).