ORIGINAL ARTICLE

Targeting integrin beta 1 using anti-tumorigenic quinazoline derivative in inhibition of glioblastoma cell invasion and survival: An in vitro preclinical study

Safdar Jawaid[1], Debatosh Majumdar[2], Saswati Banerjee[3], Saroj Pramanik[4], Santosh Mandal[5], Indrajit Chowdhury[6], Tapan K Khan*[1]

[1] *BioImaginix LLC, Morgantown, WV, United States*
[2] *GlycoMimetics, Inc., Rockville, MD, United States*
[3] *Department of Physiology, Morehouse School of Medicine, Atlanta, GA, United States*
[4] *Department of Biology, Morgan State University, Baltimore, MD, United States*
[5] *Department of Chemistry, Morgan State University, Baltimore, MD, United States*
[6] *Department of Obstetrics & Gynecology, Morehouse School of Medicine, Atlanta, GA, United States*

Received: September 3, 2025 **Accepted:** October 29, 2025 **Online Published:** November 7, 2025
DOI: 10.5430/jst.v15n1p32 **URL:** https://doi.org/10.5430/jst.v15n1p32

ABSTRACT

Objective: Glioblastoma (GBM) is the most common and malignant brain tumor, with a ∼14.5 months median survival rate without disease-modifying curative treatment. After surgical resection, radiation, and adjuvant temozolomide (TMZ) chemotherapy is the first-line treatment of GBM with adverse effects, including bone marrow suppression, genotoxicity, and teratogenicity. Here, we report a synthetic quinazoline derivative that inhibits the growth of GBM cells as a new therapeutic approach.

Methods: A potent quinazoline derivative (6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c] quinazoline) was synthesized by one chemical step with 90% yield, followed by in vitro testing in GBM and neuroblastoma cells.

Results: The in vitro studies showed that the quinazoline derivative is highly specific by decreasing GBM cell invasion while inducing cell death and inhibiting the cellular invasion in the three-dimensional Matrigel matrix. This compound is highly specific to GBM cell death compared with other cells. Synthetic quinazoline derivative is non-toxic to normal non-tumorigenic cells but toxic to cancerous cells. Under these experimental conditions, quinazoline derivatives caused inhibition of beta-1 integrin, which is an important cell adhesion molecule required for tumor cell invasion and metastasis, with extracellular matrix-mediated interactions. Furthermore, a synthetic quinazoline derivative decreases oncogenic PKC-epsilon activity in neuroblastoma cells.

Conclusions: These studies suggest that a synthetic quinazoline derivative may treat GBM effectively alone or combined with other chemotherapeutic/immunotherapeutic agents.

Key Words: Glioblastoma, Integrin β1, PKC-epsilon, Quinazoline, Temozolomide

*Correspondence: Tapan K Khan; Email: khantapan67@gmail.com; Address: BioImaginix LLC, Morgantown, WV 26508, United States.

1. INTRODUCTION

Glioblastoma (GBM) is the most common and malignant (stage IV brain tumors) among all brain tumors (∼50% of brain tumors). The median survival rate is ∼14.5 months with no disease-modifying curative treatment. Radiation and adjuvant temozolomide (TMZ) chemotherapy after the surgical resection is the first-line treatment of GBM. Common side effects of TMZ are bone marrow suppression, genotoxicity, and teratogenicity. The mechanism of action of TMZ damages GBM cells by DNA methylation and triggers the death of GBM cells. DNA damage is not specific to GBM cells. As a result, it is genotoxic. Studies found that 60%-75% of cases have no benefit from TMZ chemotherapy.[1] FDA-approved immunotherapeutic agent Bevacizumab (Avastin, Genentech) produced some improvement, including cognitive benefit,[2] but 40% of patients developed therapeutic resistance in a phase II clinical trial.[3]

In cell adhesion dynamics, integrins and extracellular matrix (ECM) mediate interactions required for tumor cell invasion and metastasis. Since the interaction of integrin beta 1 (β1) and ECM protects glioma cells, drug-induced anoikis[4] targeting integrin β1 has potential as an antiangiogenic therapy for glioblastoma.[5] The protein kinase C (PKC) signaling pathways are involved in glioma cells' aggressive behavior. Oncogenic PKC-epsilon (PKCε) is highly expressed in GBM.[6] PKCε takes part in various cellular signaling pathways that have been implicated in the progression of cancer, including GBM. These pathways regulate cell cycle progression, anti-apoptotic signaling, and the promotion of angiogenesis. Dysregulation of these processes can contribute to the development and progression of glioblastoma. Overexpression of PKCε, in general, contributes to the uncontrolled growth and survival of cancer cells, including glioblastoma. It contributes to glioma genesis and induces necrosis, autophagy, and apoptosis after the knockdown of PKCε in glioma cells to necrosis, autophagy, and apoptosis.[7,8]

Heterocyclic compounds such as quinazoline derivatives have enormous potential in modern medicinal chemistry as anti-cancer therapies. Depending on their chemical structures, quinazolines act as inducers and inhibitors of necrosis, autophagy, and apoptosis.[9] Necrosis, autophagy, and apoptosis-inducing quinazolines have potential as antitumorigenic agents. Cytotoxicity and genotoxicity of quinazolines are significant problems in cancer therapy. Here, we report the discovery and optimization of a synthetic quinazoline derivative that inhibited glioblastoma cell invasion and survival. Quinazoline moieties and conjugates help inhibit the growth of brain tumor cells through human epidermal growth factor (EGF) and CDK5.[10,11] Recent patents on the anti-cancer effect of quinazolines were reviewed by Ravez et al.[12] We have developed a synthetic agent that induces GBM cell death by reducing cellular adhesive proteins such as β-integrin and inhibiting the oncogenic activity of PKCε. Moreover, this compound is non-toxic to human primary skin fibroblasts.

2. METHODS

2.1 The design and synthesis of the synthetic quinazoline derivative

The chemical formula of the synthetic quinazoline derivative is shown in Figure 1. Synthesis of [6-(2-pyridinyl)-5,6-dihydrobenzimidazo[1,2-c] quinazoline]: The compound was synthesized as exactly reported in a peer-reviewed journal.[13] Ethanolic solution of 2-(2-aminophenyl) benzimidazole, (2.09 g, 0.0 mmoL) was added to pyridine-2-carboxaldehyde (1.07 g, 10.0 mmoL) in ethanol (25 mL) at room temperature. After refluxing in ethanol for four hours, a yellow-colored solution was obtained. The compound was precipitated as a white-colored crystalline solid through slow evaporation of the solvent. Crystal formation and its purity were checked as reported by Sen et al.[13] The compound [6-(2-pyridinyl)-5,6-dihydrobenzimidazo[1,2-c] quinazoline is referred to as Compound 1 in the text.

2.2 Cell lines and culture conditions

Human glioblastoma cells. The human GBM cell line T98-G (ATCC CRL-1690) was obtained and authenticated by the American Type Culture Collection (Cat# 30-2003; ATCC, Manassas, VA). Human mesenchymal subtype glioblastoma cell line, U3035, was received from the Human Glioblastoma Cell Culture (HGCC) Biobank, Uppsala University (Sweden). Cells were cultured and maintained in low-glucose Dulbecco's Modified Eagle Medium (DMEM) (ThermoFisher Scientific) supplemented with 10% fetal bovine serum (FBS, ThermoFisher Scientific) at 37°C in a 5% CO_2 humidified atmosphere and passed in less than six months.

Human neuroblastoma cells (Human SH-SY5Y, Sigma-Aldrich) were cultured in 45% F12K, 45% Modified Eagle Medium (MEM), supplemented with 10% FBS in a tissue culture incubator (37°C, 5% CO_2, 90% humidity). Primary skin fibroblasts. Primary skin fibroblasts were obtained from the Coriell Institute for Medical Research (Camden, NJ; Cat#AG09555; the Aging Cell Culture Repository of the National Institute on Aging) and maintained in DMEM with low glucose (Invitrogen, Grand Island, NY) supplemented with 10% FBS in 6-well culture plates (37°C, 5% CO_2, 90% humidity) until reaching 90%-100% confluence. In addition, cells were treated with either vehicle, 50 and 100 μM quinazoline derivative, and propentofylline. The condition of the

cultured cells was monitored with an inverted cell culture microscope (Westover Digital AMID Model 2000, Westover Scientific, Bothell, WA), controlled by a computer, and images were captured with image acquisition software (Micron 2.0.0, Westover Scientific).

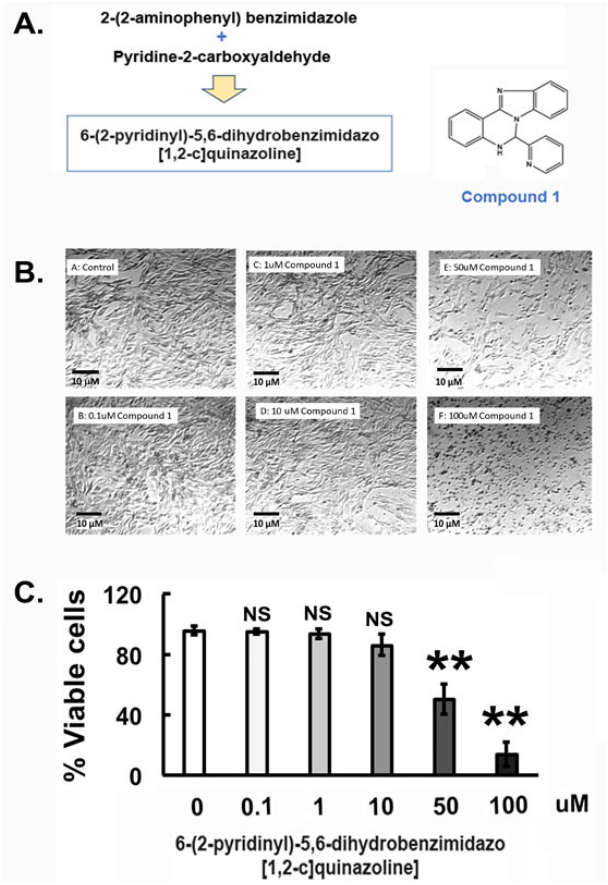

Figure 1. Synthetic quinazoline derivative synthesis and its effect on cell death

*A. Chemical structure of the synthetic quinazoline derivative. Compound 1: 6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c]quinazoline. B. Synthetic quinazoline derivative (Compound 1: 6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c]quinazoline) induces cell non-viability in Glioblastoma (GBM) cells. Cells were treated with different concentrations of Compound 1 (0.1, 1, 10, 50, 100 µM) or vehicle (control). Representative phase photomicrographs of live cells were taken under an inverted microscope (Bar = 10 µm). C. Concentration dependence of cell viability of human glioblastoma (GBM) cells by synthetic quinazoline derivative (Compound 1: 6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c]quinazoline). The bar graph represents the percentage of cells displaying nuclear morphological changes during cell non-viability. Compound 1 treatments (3 days) induce cell non-viability in 50 and 100 µM concentrations. All bar graphs represent the mean ± SEM of results from three individual experiments (n = 3). Asterisks (*) represent an unpaired Student's t-test, **p ≤ .001. NS: Non-significant.*

2.3 Reagents and antibodies

Propentofylline (molecular weight 306.366; [1-(5O-oxohexyl)-3-methyl-7-propylxanthine) was purchased from Sigma-Aldrich (St. Louis). Propentofylline and the synthetic quinazoline derivative were dissolved in DMSO (30–60 mM, depending on solubility). Aliquots were stored at –20°C. Stock solutions were dissolved in a culture medium at different concentrations just before each experiment. Rabbit monoclonal anti-Integrin β1 [CD29] antibody (Cat# 04-1109) was purchased from Millipore (Temecula, CA). Anti-tubulin III antibody (Cat# 79-720) was purchased from ProSci (Poway, CA). Antibody for PKCε (C-15) (Santa Cruz Biotechnology, Santa Cruz, CA; Cat No: sc-214) was used to detect PKCε and has been recommended for the detection of PKCε of human origin by western blotting and immunofluorescence analysis by the manufacturer.

2.4 Cell viability test by Trypan blue assay

A trypan blue exclusion test was conducted to determine the percentage of viable cells present in a cell suspension after a specific treatment. After each treatment, T98-G cells were removed from the 6-well cell culture plates by trypsinization and washed twice with PBS (1X). Freshly prepared Trypan blue solution (0.4%) was added to cell suspensions at a 1:1 ratio. A drop of the trypan blue/cell mixture was kept on a hemocytometer, and several viable/non-viable T98-G cells were counted under an inverted cell culture microscope (Westover Digital AMID Model 2000, Westover Scientific), controlled by a computer and images were captured using image acquisition software (Micron 2.0.0, Westover Scientific). The percentage of viable cells was calculated as follows: viable cells (%) = (total number of viable cells per ml of aliquot) / (total number of cells per ml of aliquot) × 100. A significant number of cells were lost in the case of prolonged Compound-1 treatment group.

Three-dimensional Matrigel basement membrane preparation for testing GBM cell invasion. The details of the preparation of the 3-dimensional Matrigel matrix have been reported by Chirila et al.[14] Growth-factor reduced Matrigel (BD Biosciences) was thawed at 4°C on ice for 30 min before use, or the stock bottle of Matrigel™ was thawed overnight at 4°C. Matrigel forms a semisolid membrane at room temperature. Therefore, it is essential to keep the Matrigel and all components used to make the solution cold/on ice. Equal volumes of the thawed Matrigel and cold DMEM were mixed to create a 1:2 dilution. To form a 3-dimensional Matrigel basement membrane, 1.5 mL BD Matrigel mixture (previously prepared 1:1 mixture of BD Matrigel and DMEM) per well (12-well plate) was added, and the homogeneity of the gel on the surface of the cell culture plates was tested un-

der the inverted microscope by checking for any bubbles on the 3-dimensional basement membrane. The 12-well plates were placed at 37°C for 30 minutes to make a semisolid 3-dimensional matrix. The T98-G cell suspensions (∼50 cells/mm^3) were added on top of the 3-dimensional Matrigel basement membrane. Images were taken under an inverted microscope (Westover Digital AMID Model 2000, Westover Scientific, Bothell, WA) equipped with Micron 2.0.0 image acquisition software. The experiment was repeated with Human mesenchymal subtype glioblastoma cell lines, U3035 (see Supplemental Figure S1).

2.5 Western blot analysis

Protein lysates (20 μg of protein each) were boiled in 2 $\times$ Laemmli buffer for 10 min in boiling water. Proteins were separated by electrophoresis using a 4%–20% gradient Tris-Glycine gel. Separated proteins were transferred to nitrocellulose membranes, and the membranes were blocked in 2% BSA (BSA dissolved in 1 $\times$ PBS) at room temperature (RT) for 1–2 hours. Membranes were then incubated with monoclonal rabbit anti-Integrin β1 [CD29] clone (1:1,000) (Cat#04-1109; Millipore), anti-PKCε, anti-β-tubulin antibody (1:10,000), and anti-β-actin antibody (1:1,000), respectively, for an overnight at 4°C. Membranes were washed three times with standard immunoblot washing buffer (TRIS-buffered Saline, pH 7.4; Alfa Aesar, Ward Hill, MA) and incubated with alkaline phosphatase-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) at a 1:10,000 dilution for 1–2 hours. Membrane fractions were washed three times with a standard immunoblot washing buffer and developed using the 1-step NBT-BCIP substrate (Thermo Scientific, Rockford, IL). Signal intensities of the images were recorded in the ImageQuant RT-ECL (GE Life Sciences, Piscataway, NJ), and densitometric quantification was performed using the IMAL software (Blanchette Rockefeller Neurosciences Institute at West Virginia University, Morgantown, WV). Intensities of β-integrin and PKCε signals were normalized against β-tubulin/ β-actin for each lane.

2.6 PKCε activity measurement

SH-SY5Y cells were grown to ∼90% confluent in culture plates and treated with 0.0 M, 1.0 nM, and 1 μM concentrations of the synthetic quinazoline derivative Compound overnight. The next day, culture media were removed from the culture plates well using vacuum suction and washed three times with 1 mL PBS at 4°C on ice. The PBS was removed completely from the wells using vacuum suction. Cells were lysed by adding 100 μL homogenizing buffer. The homogenizing buffer contains 10 mM Tris, pH 7.4, 1 mM PMSF, 50 mM NaF, 1mM EGTA, 10 μL/ml of Halt protease, and a phosphatase inhibitor cocktail. Cells were homogenized into a homogenizing buffer, collected in a 1 mL Eppendorf tube, and sonicated on a tip sonicator. The cell lysates were transferred to ultracentrifuge tubes. The cell lysates were centrifuged at 40,000 rpm for 10 minutes at 4°C. The soluble fraction was collected as a cytosol fraction in 0.5 mL tubes on ice. The individual tube pellets were homogenized into a homogenizing buffer containing 1X Triton X-100. It was called a membrane fraction. The membrane fractions were sonicated and stored at 4°C. The levels of PKCε in each membrane and cytosol fractions were measured by Western blot analysis using the PKCε antibody. The PKCε levels were quantified using densitometry from the Western blot signals. The PKCε activity was measured by the translocation ratio of the levels of PKCε in the membrane and cytosol fraction. The same amount of protein was added as the loading control. Since we have manipulated cell lysates, a specific loading control would not work in this case.

3. Statistical analysis

All experimental data are expressed as mean $\pm$ SEM of three independent experiments (n = 3). Statistical analysis was carried out by unpaired t-test, one- or two-way ANOVA using SPSS version 11.0. Multiple comparisons were made by the Newman-Keuls test. The differences were significant at $p \leq .05$.

4. Results

4.1 Synthetic quinazoline derivative (Compound 1: 6-Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c]quinazoline) induces cell non-viability in Glioblastoma (GBM) cells

The synthetic quinazoline derivative was designed and synthesized as shown in Figure 1A. To examine whether Compound 1 plays a direct role in GBM cell non-viability, GBM cells (T98-G) were cultured in 6 wells until ∼90% confluent. Cells were then treated with different concentrations of Compound 1 (0.1, 1, 10, 50, 100 μM) or vehicle (control). After three days under treatment at 50 and 100 μM concentrations, Compound 1 induced significant cell non-viability compared to the control group (see Figure 1B and 1C). Cell viability was tested by the trypan blue exclusion assay. A similar result was obtained in the cell viability assay. Cell viability was significantly reduced by the treatment of 50 and 100 μM concentrations in the Compound 1 group (see Figure 1C).

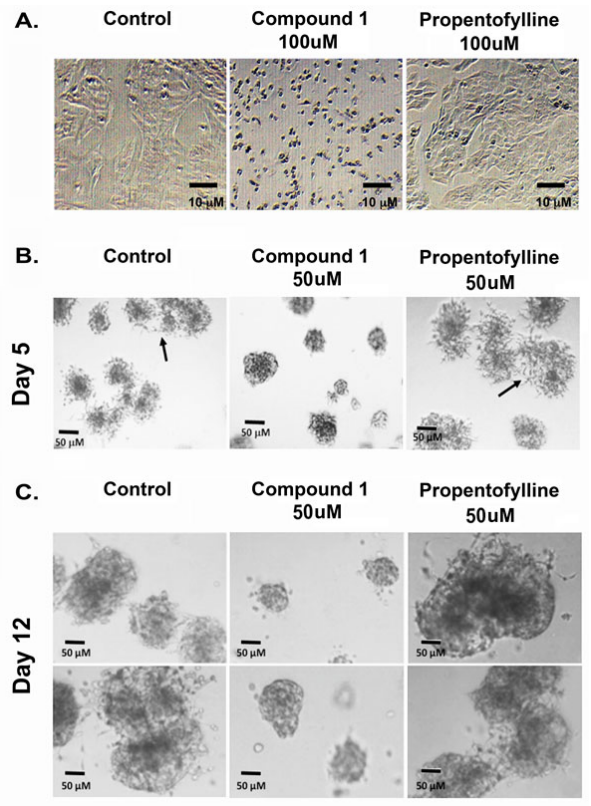

Figure 2. Anti-tumorigenic quinazoline derivative

A. Comparative intervention of induction of cellular non-viability in GBM cells by synthetic quinazoline derivative (Compound 1) versus Propentofylline. Compound 1 is more potent for inhibiting GBM survival than Propentofylline. Propentofylline is a xanthine derivative that inhibits phosphodiesterase. (Bar = 10 μm). B. Synthetic quinazoline derivative, Compound 1, decreased GBM cell invasion in a 3-dimensional Matrigel matrix. GBM cells form a 3-spheroid tumor-like biological entity in three-dimensional space. Arrows indicate invasive cells in model tumor 3-D spheroids. On the 5th day, there were marked differences in 3-D spheroid size and number of invasive cells by treatment with the most potent quinazoline Compound, control (Vehicle), and Propentofylline Compound 1 treatments reduce the model spheroid size, and cells do not come out of the spheroids because of reduced cell invasion. Arrows indicate invasive cells; Bar = 50 μm. C. Long-term effects of the synthetic quinazoline derivative, Compound 1, on GBM cell invasion in 3-dimensional Matrigel matrix. GBM cells form a spheroid tumor-like biological entity in three-dimensional space and become bigger without treatment of Compound 1 within days and become smaller or dead at 12 with treatment. Propentofylline treatment has no effect in reducing tumor-like 3D spheroid size and cell invasion.

4.2 A comparative intervention of induction of cellular non-viability in GBM cells by synthetic quinazoline derivative (Compound 1) versus Propentofylline

Based on our previous studies, we used propentofylline, a xanthine derivative that inhibits phosphodiesterase and glioblastoma cell invasion and survival by targeting the TNFRSF19 (TROY),[15] to conduct a comparative study of Compound 1 versus propentofylline. Propentofylline does not affect GBM cell growth and proliferation in the concentration range 0-50 μM.[15] We also found similar results in propentofylline-treated GBM cells. As shown in Figure 2A, Compound 1 induced cellular non-viability in GBM cells.

4.3 Synthetic quinazoline derivative, Compound 1 decreases GBM cell invasion in a 3-dimensional Matrigel matrix

GBM cells were incubated in a 3-dimensional Matrigel matrix. Multicellular spheroids were formed after three days of incubation (see Figure 2B). Cellular invasiveness was monitored in multicellular spheroids of GBM embedded in a 3D Matrigel matrix. Invasive cells were sprouting outside the spheroid in the case of control and Propentopfylline at 50 μM concentration (see Figure 2B) treatment. In contrast, there were reduced invasive GBM cells when treated with 50 μM Compound 1 (see Figure 2B).

In further studies, we evaluated the long-term effects of the synthetic quinazoline derivative, Compound 1, on GBM cell invasion in a 3-dimensional Matrigel matrix. As shown in Figure 2C, GBM cells form a spheroid tumor-like 3-D biological entity in three-dimensional space. Without treatment, they become larger within days, whereas with treatment, they become smaller or die by day 12. Compound 1 treatments unequivocally reduce the model spheroid size, and cells did not come out of the spheroids because of reduced cell invasion. Propentofylline treatment did not reduce the 3-D spheroid size and cell invasion under these experimental conditions. These results suggest that the current quinazoline derivative compound 1 is more potent than Propentofylline in inducing cell non-viability in GBM cells. Similar results were observed in the case of Human mesenchymal subtype glioblastoma cell lines, U3035 (see Supplementary Figure S1).

4.4 Synthetic quinazoline derivative, Compound 1 decreases cell invasion by decreasing β1 integrin (CD29) levels

We aim to investigate the ability of a synthetic potent quinazoline derivative to reduce β1 integrin levels, given that β1 integrin levels are upregulated in GBM cells that have acquired resistance to bevacizumab (Avastin; Genentech) therapy.[5] Treatment of GBM by the synthetic quinazoline derivative (Compound 1) decreased β1 integrin (CD29) levels (see Figure 3A-B). The synthetic quinazoline derivative decreased the levels of β1 integrin significantly ($p < .02$). We also compared the effectiveness of reducing β1 integrin level

with Propentofylline in the same concentration. Propentofylline could not reduce β1 integrin level ($p < .04$) (see Figure 3C). Synthetic quinazoline derivative decreases oncogenic PKCε activity in neuroblastoma cells.

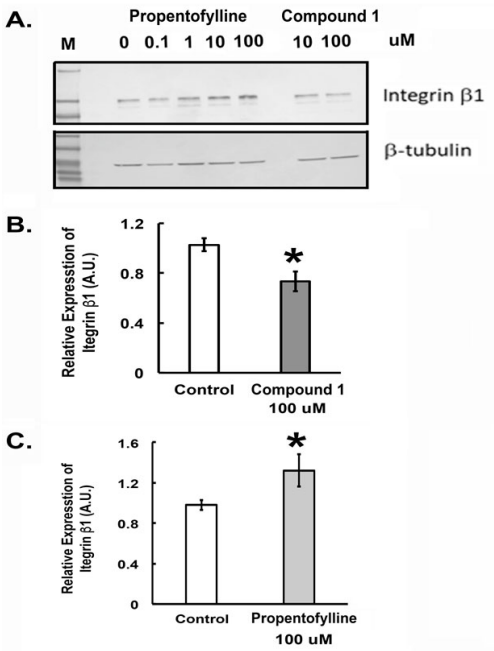

Figure 3. Quinazoline derivative (Compound 1: -Pyridin-2-yl-5,6-dihydro-benzo[4,5] imidazo[1,2-c]quinazoline) decreases glioblastoma cell invasion by decreasing β1 Integrin (CD29) levels

*Representative WB analysis of protein levels of β1 Integrin in GBM cells treated with or without Compound 1 and Propentofylline for the days indicated in micrographs. Equal amounts of protein were applied to each lane. Relative expression levels of β1 Integrin are normalized with β-tubulin as a loading control in Western blot analyses. The bar diagram represents the densitometric analyses of β1 Integrin in WB of three independent experiments. Data is given as mean $\pm$ standard error of the mean (SEM). *$p \leq .05$ with the control group (Control: 1.018054591, 0.896338962, 1.027069558; Average (Av) = 0.980487704; Standard deviation (Stdev) = 0.073014214; SEM = 0.051636643; 100 μM Propentofylline: 1.053645538, 1.42589523, 1.476853852; Av = 1.318798208; Stdev = 0.231038198; SEM = 0.163393351; 100 μM Compound 1: 0.864960412, 0.662476842, 0.675535689; Av = 0.734324314; Stdev: 0.113322443; SEM = 0.08014317; Student's t-test p values: Control vs. 100 μM Propentofylline = 0.036446086; Control vs. 100 μM Compound 1: 0.017046354). β1 Integrin band decreased in Western blot analyses, both in normal culture (A), and serum-free condition, by the treatment of Compound 1 and Propentofylline. Propentofylline treatment produces reverse effects (A). Compound 1 significantly decreases integrin β1 expression level (B) ($p < .02$) (10 μM), whereas propentofylline significantly increases integrin β1 expression level (C) ($p < .04$) at similar concentrations. SEM is calculated from three independent measurements*

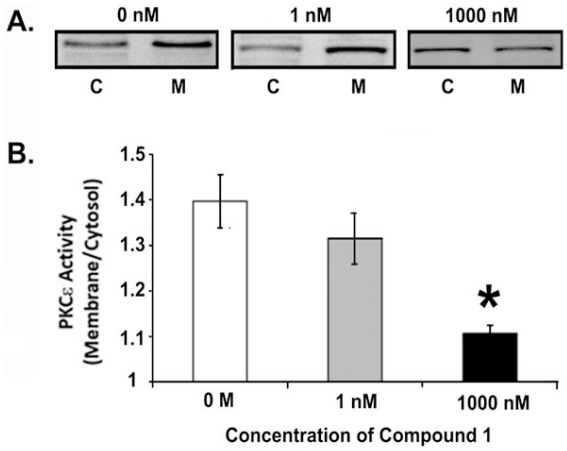

Figure 4. Synthetic quinazoline derivative, Compound 1, decreases oncogenic PKCε activity in neuroblastoma cells

A. The translocation ratio of the levels of PKCε in membrane and cytosol fractions of GBM cells were measured by Western blot analysis using PKCε antibody. B. The bar diagram represents the densitometric analyses of PKCε in membrane (M) and cytosolic (C) fractions of cells of three independent experiments. SEM is calculated from three independent measurements. (M/C: 0 M Compound1: 1.329159935, 1.373251029, 1.486785495; Mean = 1.39639882; Standard deviation = 0.081322318 Standard error of mean (SEM) = 0.0575; 1 nM Compound 1: 1.343909555; 1.376324706, 1.226694915 Mean = 1.315643059; Standard deviation = 0.078717944; SEM = 0.055670399; 1,000 nM: 1.133271823, 1.08418706, 1.103095421; Mean = 1.106851435 0.017508489; Standard deviation= 0.024757004; SEM = 0.002064915; Student's t-test for 0 M Compound1vs 1,000 nM Compound1 p-value = .002064915)

PKCε is an oncogenic survival kinase for GBM cells.[16] Therefore, we tested whether the synthetic quinazoline derivative can decrease oncogenic PKCε activity in neuroblastoma, SHSY-5Y cells. As shown in Figure 4A-B, Compound-1 at higher concentration significantly decreased the PKCε activity in membrane/cytosolic fraction in non-GBM cells (SH-SY5Y), whereas Compound-1 at lower concentration had no significant effects on the PKCε activity in the membrane/cytosolic fraction. These results suggest that Compound-1 is potent in decreasing oncogenic PKCε activity in other cells, such as neuroblastoma, under Quinazoline derivative treatment. We found that the quinazoline derivative, Compound 1, was non-toxic to cultured primary human skin fibroblast cells isolated from healthy individuals. There was no primary human skin fibroblast cell death observed after three days of continuous treatment with 100 μM Compound 1 (see Supplementary Figure S2). Our study found that Compound 1 was a more potent inhibitor of GBM cell invasion than Propentofylline (see Figure 2 and Supplemental Figure S1).

5. DISCUSSION

In the current study, we have demonstrated a potential role for a quinazoline derivative against GBM. Quinazoline derivatives have enormous potential in modern medicinal chemistry as anti-cancer therapy. Depending on their chemical structures, quinazolines act as inducers and inhibitors of necrosis, autophagy, and apoptosis.[9] Necrosis, autophagy, and apoptosis-inducing quinazolines have potential as anti-tumorigenic agents. Cytotoxicity and genotoxicity of quinazolines are significant challenges in cancer therapy. Propentofylline (molecular weight 306.366) is a synthetic methyl xanthine derivative recently shown to decrease GBM cell invasion and survival.[15] Previously, it has been tested as a therapeutic agent for Alzheimer's disease. However, it was ineffective in inducing necrosis, autophagy, and apoptosis in GBM cells.[17] In the present study, we further compare the ability to induce necrosis, autophagy, and apoptosis, and reduce cellular invasion of GBM by synthetic moieties of quinazoline with propentofylline. Propentofylline did not induce cell non-viability in GBM cells in culture conditions. At the same time, two of the quinazoline moieties caused significant cell non-viability in GBM cells at 50-100 μM concentrations. Interestingly, the mechanical properties of cell invasion of patient-derived primary glioblastoma cells have been studied recently in a 3-D Matrigel matrix.[18] Several studies showed that propentofylline decreases GBM cell invasion and inhibits GBM survival.[15,17]

Integrins have an important role to play in cell invasion in tumorigenic environments. They are receptors of various ECM components. β1 integrin was found to be the main component of the signaling pathway stimulating glioma cell invasion.[19] Moreover, the role of β1 integrin has been implicated in tumor vascularization both by VEGF-dependent and VEGF-independent manner of promoting endothelial cell migration.[20,21] β1 integrin levels were upregulated in GBM cells that acquired resistance to bevacizumab (Avastin; Genentech) therapy.[5] We intend to find the capability of the most potent quinazoline derivative to reduce β1 integrin levels. The most potent quinazoline derivative decreased β1 integrin levels significantly. We also compared the effectiveness of lowering β1 integrin levels with propentofylline in the same concentration. Propentofylline was not able to reduce β1 integrin levels. The deletion of β1 Integrin reduced tumorigenesis in an in vivo xenograft tumor growth animal model.[22] Integrins are a class of molecules involved in GBM cell adhesion, migration, and cellular invasion.[23] These transmembrane receptors facilitate extracellular matrix adhesion by binding with glycoproteins such as laminins and collagens. β1 Integrin regulates adhesion, migration, and invasion of all kinds of cancer cells. Integrin also increases the chances of survival of malignant cells by increasing anti-apoptotic proteins such as Bcl-2 and FLIP. Our current studies have demonstrated that Compound 1 significantly reduced expression levels of β1 Integrin, whereas propentofylline has increased expression levels of β1-Integrin in GBM cells at similar concentrations (see Figure 3). In addition, PKCε has been implicated in the regulation of β1 Integrin expression in different cancer cells, such as renal cancer,[24] metastatic cancer cells,[25] and lung cancer.[26] We intended to investigate the anti-tumorigenic effects of Compound 1, specifically its impact on PKCε activity. Generally, well-established cell lines are used for this kind of study. Therefore, we used well-established SH-SY5Y cell lines. Using different glioblastoma (GBM) cell lines for studying PKC activity is a necessary practice due to the high molecular and genetic variability of GBM. Different cell lines, which are often derived from different patients, can show distinct differences in their genetic profiles, cell behaviors, and signaling pathways involving PKC. Studying β-integrin protein levels is crucial because they, not RNA levels, determine the molecule's actual function and location in the cell. Discordance often exists between RNA and protein concentrations due to complex post-transcriptional and post-translational regulatory mechanisms. The current study has established a lead compound of quinazoline derivative with a concentration range of 50-100 μM. Our future study will screen the most potent derivatives against the exact derivative. The present findings may support the treatment of GBM patients by reducing cell invasion and cell death, either through a quinazoline derivative that directly induces cell non-viability or via antibody-drug conjugation (ADC), specifically anti-TNFRSF19-quinazoline derivative conjugation. TNFRSF19 (a TNF Receptor Superfamily Member 19), also known as TROY, is associated with colorectal cancer[27] and nasopharyngeal carcinoma,[28] including GBMs.[15,29] Thus, ADC will help with more targeted therapy in our future studies.

Compound 1 remotely resembles the xanthine structure (e.g., propentofylline); thus, we compared it with propentofylline effects since propentofylline affects TNFRSF19 expressed on GBM cells and has been suggested as a therapeutic target for GBM.[15] However, propentofylline does not affect GBM cell growth in the concentration range 0-50 μM.[15] Here, Compound 1 reduced the cell viability of GBM. Propentofylline is unable to show any such activities against GBM cells, but Compound 1 did, because it acts through the integrin β1. Propentofylline and Compound 1 act by two different pathways. We intend to establish that the current quinazoline derivative would be a better option to induce GBM cell death.

Compound 1 has not been tested to evaluate whether it can cross the blood-brain barrier (BBB). We intend to study it in

our future studies. However, Compound 1 can be used to induce GBM cell death after surgical resection as a chemotherapeutic agent. Even if it cannot pass the BBB, it can be used during surgical resection in the future. The role of Integrin $\beta 1$ is indeed linked with angiogenesis in glioblastoma. For example, integrins are considered membrane markers for the GBM-initiating cells subpopulation.[30] The studies proved that Integrin βs are highly expressed in GBM and should be inhibited to reduce GBM cell invasion. Therefore, the current quinazoline derivative can be used to reduce the invasion of GBM by reducing integrin β. Primary skin fibroblasts were used to show that Compound 1 is not toxic to normal human cells. Therefore, it can selectively induce anti-tumorigenic activity only in cancerous cells, leaving normal cells aside. In our studies, the synthetic quinazoline moiety, Compound 1, was non-toxic to cultured primary human skin fibroblast cells isolated from healthy individuals. There was no primary human skin fibroblast cell death observed after three days of continuous treatment with 100 μM Compound1. Our study found that Compound 1 was a more potent inhibitor of GBM cell invasion than Propentofylline (see Figure 2 and Supplemental Figure S1). Inhibition of β-integrin expression appeared statistically significant at 100 μM, although it was not completely inhibited. We are looking for other significant pathways as an alternative approach, e.g., TNFRSF-19, in our future studies. TNFRSF-19 is highly expressed in GBM. It plays a vital role in GBM invasion and resistance.

6. Conclusions

Our current studies support a new role for quinazoline derivative (Compound 1) in GBM cells as an anti-invasive and pro-apoptotic molecule by the inhibition of $\beta 1$ Integrin. Interestingly, the quinazoline compound is non-toxic to normal skin fibroblasts; however, it is toxic to the GBM cells. The study demonstrated cell viability by one assay (trypan blue method) and model tumor 3-D spheroid formation by another assay (Matrigel assay), and more stress was given to mechanistic aspects, like inhibition of angiogenic $\beta 1$ Integrin and oncogenic PKCε. In the future, we will extend our current studies in xenograft nude mice models to validate the observed effects.

Acknowledgements

This study was supported in part by intramural BioImaginix, LLC Grants (TK). Saswati Banerjee and Indrajit Chowdhury are supported in part by National Institutes of Health Grant 1SC1 GM130544. Dr. Christopher P. Cifarelli, Department of Neurosurgery, West Virginia University School of Medicine, Morgantown, WV, shared Human mesenchymal subtype glioblastoma cell lines, U3035, received from the Human Glioblastoma Cell Culture (HGCC) Biobank from Uppsala University (Sweden).

Authors contributions

TK conceived and supervised the study; SJ and TK did lab work; TK, DM, SB, SP, SM, and IC analyzed data; TK and IC wrote the manuscript; TK and IC made manuscript revisions.

Funding

The research was partly supported by the Intramural Research Program of BioImaginix LLC (T.K.).

Conflicts of Interest Disclosure

TK has a certain number of shares in BioImaginix, LLC. The other authors have nothing to disclose.

Informed consent

All authors of the article hereby provide consent for the publication of the manuscript. Authors acknowledge that the manuscript will be freely available online and anybody can access. We confirm that we have been allowed to view the manuscript before publication.

Ethics approval

The Publication Ethics Committee of the Sciedu Press. The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

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

Data sharing statement

No additional data are available.

REFERENCES

[1] Chamberlain MC. Temozolomide: therapeutic limitations in the treatment of adult high-grade gliomas. Expert Rev Neurother. 2010; 10(10): 1537-1544. PMid: 20925470. https://doi.org/10.1586/ern.10.32

[2] Kienast Y, Winkler F. Therapy and prophylaxis of brain metastases. Expert Rev Anticancer Ther. 2010; 10: 1763-1777. PMid: 21080803. https://doi.org/10.1586/era.10.165

[3] Vredenburgh JJ, Desjardins A, Herndon JE II, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clin Cancer Res. 2007; 13: 1253-1259. PMid: 17317837. https://doi.org/10.1158/1078-0432.CCR-06-2309

[4] Uhm JH, Dooley NP, Kyritsis AP, et al. Vitronectin, a glioma-derived extracellular matrix protein, protects tumor cells from apoptotic death. Clin Cancer Res. 1999; 5: 1587-1594.

[5] Carbonell WS, DeLay M, Jahangiri A, et al. β1 integrin targeting potentiates antiangiogenic therapy and inhibits the growth of bevacizumab-resistant glioblastoma. Cancer Res. 2013; 73(10): 3145-3154. PMid: 23644530. https://doi.org/10.1158/0008-5472.CAN-13-0011

[6] Sharif TR, Sharif M. Overexpression of protein kinase C epsilon in astroglial brain tumor-derived cell lines and primary tumor samples. Int J Oncol. 1999; 15: 237-43. https://doi.org/10.3892/ijo.15.2.237

[7] Basu A, Sivaprasad U. Protein kinase Cepsilon makes the life and death decision. Cell Signal. 2007; 19: 1633-1642. PMid: 17537614. https://doi.org/10.1016/j.cellsig.2007.04.008

[8] Gorin MA, Pan Q. Protein kinase C epsilon: an oncogene and emerging tumor biomarker. Mol Cancer. 2009; 8: 9-17. PMid: 19228372. https://doi.org/10.1186/1476-4598-8-9

[9] Mehndiratta S, Sapra S, Singh G, et al. Quinazolines as apoptosis Inducers and Inhibitors: A Review of Patent Literature. Recent Pat Anticancer Drug Discov. 2016; 11: 2-66. PMid: 26681186. https://doi.org/10.2174/1574892811666151218151506

[10] WO1999061428A1: Quinazolines for treating brain tumor.

[11] Peyressatre M, Arama DP, Laure A, et al. Identification of Quinazolinone Analogs Targeting CDK5 Kinase Activity and Glioblastoma Cell Proliferation. Front Chem. 2020; 8: 691. PMid: 32974274. https://doi.org/10.3389/fchem.2020.00691

[12] Ravez S, Castillo-Aguilera O, Depreux P, et al. Quinazoline derivatives as anticancer drugs: a patent review (2011 - present). Expert Opin Ther Pat. 2015; 25(7): 789-804. PMid: 25910402. https://doi.org/10.1517/13543776.2015.1039512

[13] Sen S, Mukherjee T, Sarkar S, et al. Development of a highly selective cell-permeable ratiometric fluorescent chemosensor for oxorhenium(V) ion. Analyst. 2011; 136(22): 4839-4845. PMid: 21971398. https://doi.org/10.1039/c1an15609h

[14] Chirila FV, Khan TK, Alkon DL. Spatiotemporal complexity of fibroblast networks screens for Alzheimer's disease. J Alzheimers Dis. 2013. 33(1): 165-76. PMid: 22886026. https://doi.org/10.3233/JAD-2012-120745

[15] Dhruv HD, Roos A, Tomboc PJ, et al. Propentofylline inhibits glioblastoma cell invasion and survival by targeting the TROY signaling pathway. J Neurooncol. 2016; 126(3): 397-404. PMid: 26559543. https://doi.org/10.1007/s11060-015-1981-0

[16] Okhrimenko H, Lu W, Xiang C, et al. Protein kinase C-epsilon regulates the necrosis, autophagy, and apoptosis and survival of glioma cells. Cancer Res. 2005; 65(16): 7301-7309. PMid: 16103081. https://doi.org/10.1158/0008-5472.CAN-05-1064

[17] Jacobs VL, Liu Y, De Leo JA. Propentofylline targets TROY, a synthetic microglial signaling pathway. PLoS One. 2012; 7(5): e37955. PMid: 22649568. https://doi.org/10.1371/journal.pone.0037955

[18] Grundy TJ, De Leon E, Griffin KR, et al. Differential response of patient-derived primary glioblastoma cells to environmental stiffness. Sci Rep. 2016; 6: 23353. PMid: 26996336. https://doi.org/10.1038/srep23353

[19] Paulus W, Baur I, Beutler AS, et al. Diffuse brain invasion of glioma cells requires β1 integrins. Lab Invest. 1996; 75(6): 819-826. https://doi.org/10.1097/00005072-199605000-00021

[20] Leenders WP, Kusters B, Verrijp K, et al. Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option. Clin Cancer Res. 2004; 10: 6222-6230. PMid: 15448011. https://doi.org/10.1158/1078-0432.CCR-04-0823

[21] Azam F, Mehta S, Harris AL. Mechanisms of resistance to antiangiogenesis therapy. Eur J Cancer. 2010; 46: 1323-1332. PMid: 20236818. https://doi.org/10.1016/j.ejca.2010.02.020

[22] Hou S, Isaji T, Hang Q, et al. Distinct effects of β1 integrin on cell proliferation and cellular signaling in MDA-MB-231 breast cancer cells. Sci Rep. 2016; 6: 18430. PMid: 26728650. https://doi.org/10.1038/srep18430

[23] Paolillo M, Serra M, Schinelli S. Integrins in glioblastoma: Still an attractive target? Pharmacol Res. 2016; 113(Pt A): 55-61. PMid: 27498157. https://doi.org/10.1016/j.phrs.2016.08.004

[24] Brenner W, Benzing F, Gudejko-Thiel J, et al. Regulation of β1 integrin expression by PKCepsilon in renal cancer cells. Int J Oncol. 2004; 25(4): 1157-63. PMid: 15375568. https://doi.org/10.3892/ijo.25.4.1157

[25] Ramsay AG, Marshall JF, Hart IR. Integrin trafficking and its role in cancer metastasis. Cancer Metastasis Rev. 2007; 26(3-4): 567-78. PMid: 17786537. https://doi.org/10.1007/s10555-007-9078-7

[26] Caccavari F, Valdembri D, Sandri C, et al. Integrin signaling and lung cancer. Cell Adh Migr. 2010; 4(1): 124-9. PMid: 20139694. https://doi.org/10.4161/cam.4.1.10976

[27] Nishioka M, Suehiro Y, Sakai K, et al. TROY is a promising prognostic biomarker in patients with colorectal cancer. Oncol Lett. 2018; 15(4): 5989-5994. PMid: 29556315. https://doi.org/10.3892/ol.2018.8079

[28] Deng C, Lin YX, Qi XK, et al. TNFRSF19 Inhibits TGFβ Signaling through Interaction with TGFβ Receptor Type I to Promote Tumorigenesis. Cancer Res. 2018. 78(13): 3469-3483. PMid: 29735548. https://doi.org/10.1158/0008-5472.CAN-17-3205

[29] Paulino VM, Yang Z, Kloss J, et al. TROY (TNFRSF19) is overexpressed in advanced glial tumors and promotes glioblastoma cell invasion via Pyk2-Rac1 signaling. Mol Cancer Res. 2010; 8(11): 1558-1567. PMid: 20881009. https://doi.org/10.1158/1541-7786.MCR-10-0334

[30] Malric L, Monferran S, Gilhodes J, et al. Interest of integrins targeting in glioblastoma according to tumor heterogeneity and cancer stem cell paradigm: an update. Oncotarget. 2017; 8(49): 86947-86968. PMid: 29156849. https://doi.org/10.18632/oncotarget.20372