Bavachin Suppresses Cell Growth and Enhances Temozolomide Efficacy in U-87 MG Glioblastoma Cells

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ABSTRACT
Glioblastoma multiform (GBM) is a lethal cancer affecting the central nervous system. Existing treatment methods for GBM include chemotherapy, radiation, and surgery. Temozolomide is a common chemotherapy treatment for GBM but causes unwanted side effects, as do most chemotherapy treatments. To decrease the harmful side effects of chemotherapy, research has turned to exploring natural compounds to treat cancer. In this study, the natural compound bavachin was investigated to determine cytotoxicity in GBM cells. Bavachin has been tested in many cell lines but not glioblastoma. A sulforhodamine B (SRB) assay, trypan blue counting, and a tumorsphere assay were used to test the hypothesis in this study. It was found that bavachin suppressed glioblastoma cell and tumorsphere viability, had a synergistic interaction with temozolomide, and ornithine decarboxylase was identified as a potential target *in silico*. This study demonstrated the ability of bavachin to suppress the growth of U-87 MG glioblastoma cells and enhance temozolomide efficacy. These findings demonstrated that bavachin is a potential treatment for GBM based on its ability to suppress cell growth.

KEYWORDS
Glioblastoma Multiforme; Temozolomide; Bavachin; Flavonoid; Tumorspheres; Cell Viability; Cancer; Apoptosis; *Psoralea corylifolia*

INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most aggressive cancers with a high rate of treatment resistance. Glioblastoma begins as an abnormal growth of astrocytes that eventually crowds and harms healthy tissue. Astrocytes are a type of glial cell in the central nervous system that forms scar tissue to repair the brain when damaged. Glioblastomas are classified as IV-grade astrocytoma tumors by W.H.O. due to their fast growth and are the most common malignant brain tumors in adults.[1] Although the risk of forming glioblastoma is low, in 2023 the American Cancer Society predicted 24,810 malignant tumors diagnoses and 18,990 deaths that year from glioblastoma.[1]

The known forms of treatment for GBM include chemotherapy, radiation, and surgery. Chemotherapy with temozolomide (TMZ) is commonly used to treat GBM, but GBM rarely responds to treatment.[2] TMZ is an imidazotetrazine lipophilic prodrug that can cross the blood-brain barrier and is administered orally. TMZ is activated at physiological pH through conversion to the metabolite 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC). MTIC is then hydrolyzed to produce methyldiazonium, which damages DNA by forming multiple DNA adducts. These adducts create an opportunity for mismatched base pairing.[3] While survival rates have improved, individual variability remains significant with average survival extending from ten to fourteen months post-diagnosis.[3] Resistance to TMZ develops quickly due to widespread exposure and DNA repair mechanisms. Cells contain DNA repair mechanisms that detect and correct mismatched bases to prevent apoptosis. As methyldiazonium ions are added, O6-methylguannine adducts are generated. O6-methylguanine-DNA methyltransferase (MGMT) works to counteract the damage done by O6-methylguannine.[4]

Effective treatment for GBM remains a formidable challenge in molecular oncology, with high-grade gliomas exhibiting unfavorable outcomes despite advances in diagnosis and therapy. GBM treatment is further complicated by factors such as the advanced age of onset, tumor location, and incomplete understanding of the tumor's pathophysiology.[5] Due to the limitations of current treatments, including their potential to harm healthy cells, naturally-occurring compounds are becoming a popular focus of study in many cancers including GBM. Various natural compounds such as flavonoids, polyphenolic compounds, curcumin,

icariin, and others exhibit anticancer potentials through different mechanisms such as induction of apoptosis, inhibition of angiogenesis, modulation of intracellular signaling pathways, and inhibition of cell proliferation.[6-10]

Flavonoids are found in many fruits, vegetables, and other vascular plants. While flavonoids can adversely affect bacteria, viral enzymes, and pathogenic protozoans, they show little toxicity in animals. Flavonoids have been studied as herbal medical treatments for decades and used for much longer. It has been determined that flavonoids inhibit certain enzymes, mimic some hormones (including estrogen), and scavenge free radicals.[11–13] Additionally, flavonoids inhibit malignant cell proliferation by directly inhibiting mitosis. One promising flavonoid to inhibit GBM cell growth is bavachin. It is isolated from the fruit of *Psoralea corylifolia*, Fructus Psoraleae, a legume native to China.[14] In contrast to TMZ, bavachin is a natural compound that has been shown to target cancer cells more specifically. Bavachin is currently being explored for its anti-cancer effects and has been tested in multiple cancer cell lines including oral, bone, multiple myeloma, choriocarcinoma, colorectal, and liver. In each of these studies, bavachin was shown to induce apoptosis.[6, 15–18]

This research study focused on the apoptotic effects of the flavonoid bavachin in the GBM cell line, U-87 MG. This study implemented SRB, trypan blue, and tumorsphere assays for measuring cell viability in response to bavachin treatment. Additionally, molecular docking software was used to create an *in silico* model of bavachin bound to ornithine decarboxylase. Finally, U-87 MG cells were treated with bavachin and TMZ together to evaluate the combined drug interaction. We hypothesized that bavachin would limit cell viability, inhibit tumorsphere growth, and induce apoptosis in the glioblastoma cell line, U-87 MG, by inhibiting ornithine decarboxylase.

METHODS AND PROCEDURES

Cell Culture

U-87 MG cells (ATCC, Manassas VA, USA) were cultured in Eagle's Minimum Essential Media (Corning, Corning NY, USA) with penicillin-streptomycin (200 IU/L) (Corning) and 10% fetal bovine serum (ThermoFisher Scientific, Waltham MA, USA). Cells were left to grow and proliferate for at least two days at 37 °C, 100% humidity, and 5% CO_2 before experimentation. Once cells reached 70% confluence, the cell medium was aspirated; cells were washed with warm PBS (ThermoFisher), and two mL of trypsin (ThermoFisher) were added to lift the cells for five minutes at 37 °C. The trypsin was neutralized with eight mL of media and collected cells were briefly vortexed before counting with a hemocytometer. All experiments were conducted using U-87 MG cells within one to five passages from acquisition.

SRB Viability Assay[19]

Cells (5 X 10^3) were seeded in 96 well plates (Genesee Scientific, El Cajon CA, USA) with 100 µL media and allowed to adhere overnight. Then 100 µL of media containing 2X concentration of bavachin (MedChemExpress, Monmouth Junction NJ, USA) or negative control (1% DMSO) (UFC Biotechnology, Buffalo NY, USA) were overlayed in each well for 48 hours. Each well then received 100 µL of 10% w/v cold trichloroacetic acid (ThermoFisher) and was incubated at 4 °C for one hour to fix the cells. Each well was rinsed three times with DI H_2O and stained with 100 µL 0.057% w/v sulforhodamine B (SRB, Sigma-Aldrich) for 30 minutes at room temperature in the dark. Wells were washed four times with 300 µL 1% v/v acetic acid (ThermoFisher), and the remaining SRB stain was solubilized with 200 µL of 10 mM tris buffer, pH 10.5 (ThermoFisher). Absorbance was measured at 564 nm with a SpectraMax 190 Microplate Reader (Molecular Devices, San Jose CA, USA) and normalized to the DMSO control. Cells were also imaged at 40X total magnification with an Olympus BX41 phase contrast microscope (Hachioji, Tokyo, Japan) with an AmScope HD202-MW camera (Irvine, CA USA). Some SRB assays included temozolomide (MedChemExpress) to determine drug synergy.

Trypan Blue Viability Assay

Cells (1 X 10^5) were seeded in six-well plates (Advangene Consumables, Lake Bluff IL, USA) with two mL of media in each well and incubated at 37 °C overnight to allow cells to adhere. Then, media were removed, and cells were treated with DMSO or 50 µM of bavachin. After 48 hours of treatment, the media were removed, cells were washed with PBS, lifted with trypsin for five minutes, briefly vortexed, and diluted 1:2 into 0.4% (w/v) trypan blue (Sigma-Aldrich, St. Louis MO, USA). The number of viable cells was counted with a hemocytometer.

Tumorsphere Generation

U-87 MG cells were used to generate stem cell spheroids. A low attachment plate method was used to generate spheroids.[20–22] Cells (5 X 10^2 in 200 µl media) were seeded in a Sphera low-attachment surface plate (ThermoFisher). The plate contained a hydrophilic polymer coating that lowers attachment and promotes cell aggregation. The low-attachment plate was centrifuged for five minutes at 1250 RPM. The cells were incubated at 37 °C at 5% CO_2 and 100% humidity. Plates were treated four days after seeding. Media was replenished on days 7, 10, and 12 by carefully removing 100 µl of media from each well and adding 100 µl of

fresh media to each well. Spheres were imaged on day 14 using an Olympus BX41 phase contrast microscope at 100X total magnification with an iPhone SE camera (Apple Inc., Cupertino CA, USA). The radius of each tumorsphere was measured. The radius and **Equation 1** were used to determine the volume of the spheres.

$$V = \frac{4}{3}\pi r^3 \qquad \textbf{Equation 1.}$$

Molecular Docking

Molecular docking was used to determine a possible protein target of bavachin. Free software including ZINC20, CB-Dock2, and ChimeraX were used to model and view the *in silico* model. Briefly, ZINC20 was used to provide a list of potential binding targets for bavachin (ZINC ID: ZINC15115057) using the Similarity Ensemble Approach (SEA).[23] Ornithine decarboxylase (ODC) was chosen as the target for docking with bavachin. The crystal structure for ODC was downloaded from the Protein Data Base (PDB: 1D7K) to model bavachin binding in CB-Dock2.[24, 25] Finally, the bavachin-ODC interaction was visualized using ChimeraX.[26]

Data analysis

All statistical analyses were performed using GraphPad Prism 10.3.1 and assumed that variance was similar between experimental groups. Sample sizes were a minimum of three independent experiments. Group comparisons were made by one-way ANOVA followed by the Dunnett multiple comparison test. Paired comparisons were conducted by unpaired Student t-test assuming two-tailed distribution. Data are represented as mean ± SD. Differences were considered statistically significant at $*P < 0.05$, $**P \leq 0.01$, $****P \leq 0.0001$, (ns = not significant $P \geq 0.05$) when compared to the negative control, DMSO, throughout all testing. Drug synergy was evaluated using the coefficient of drug interaction (CDI) formula (**Equation 2**). AB is the percent remaining cells of an indicated combination treatment, A is the average percent remaining cells of agent one alone, and B is the average percent remaining cells of agent two alone. A CDI of ≤ 0.7 is considered significantly synergistic; CDI = 1 is additive; CDI > 1.0 is antagonistic.[27]

$$CDI = \frac{AB}{A\ X\ B} X\ 100 \qquad \textbf{Equation 2.}$$

RESULTS

Bavachin Reduces U-87 MG Cell Viability

In this study, a sulforhodamine B (SRB) assay was performed to determine the cell viability of cell line U-87 MG after treatment with seven concentrations of bavachin over 48 hours. As seen in **Figure 1A**, bavachin inhibited cell viability in a concentration-dependent manner. Bavachin concentrations of 1, 10, and 25 μM did not have a significant impact on cell viability. However, significant cell death could be detected starting at 50 μM of bavachin. This assay was used to calculate the half maximal inhibitory concentration (IC_{50}) value, which was determined to be 46.00 μM as shown in **Figure 1B**. Therefore, 50 μM bavachin was used for the subsequent trypan blue assay.

Trypan blue only stains dead cells because it is non-permeable to the cell membrane, so it can only enter cells when they are late-stage apoptotic. This assay allowed apoptotic cells to be counted and compared to the total cells counted. Staining with trypan blue after bavachin treatment showed significant cell death and apoptosis compared to the negative control, DMSO, as seen in **Figure 1C**. Not only did we see a comparable reduction in cell viability in the trypan blue assay compared to the SRB assay, but we found the bavachin-treated group contained 18% apoptotic cells (**Figure 1D**). Phase-contrast microscopy in **Figure 1E**, **Figure 1F**, and **Figure 1G** visualized bavachin's ability to suppress growth and cause cell death at 50 μM and 200 μM compared to the control cells with only 1% DMSO.

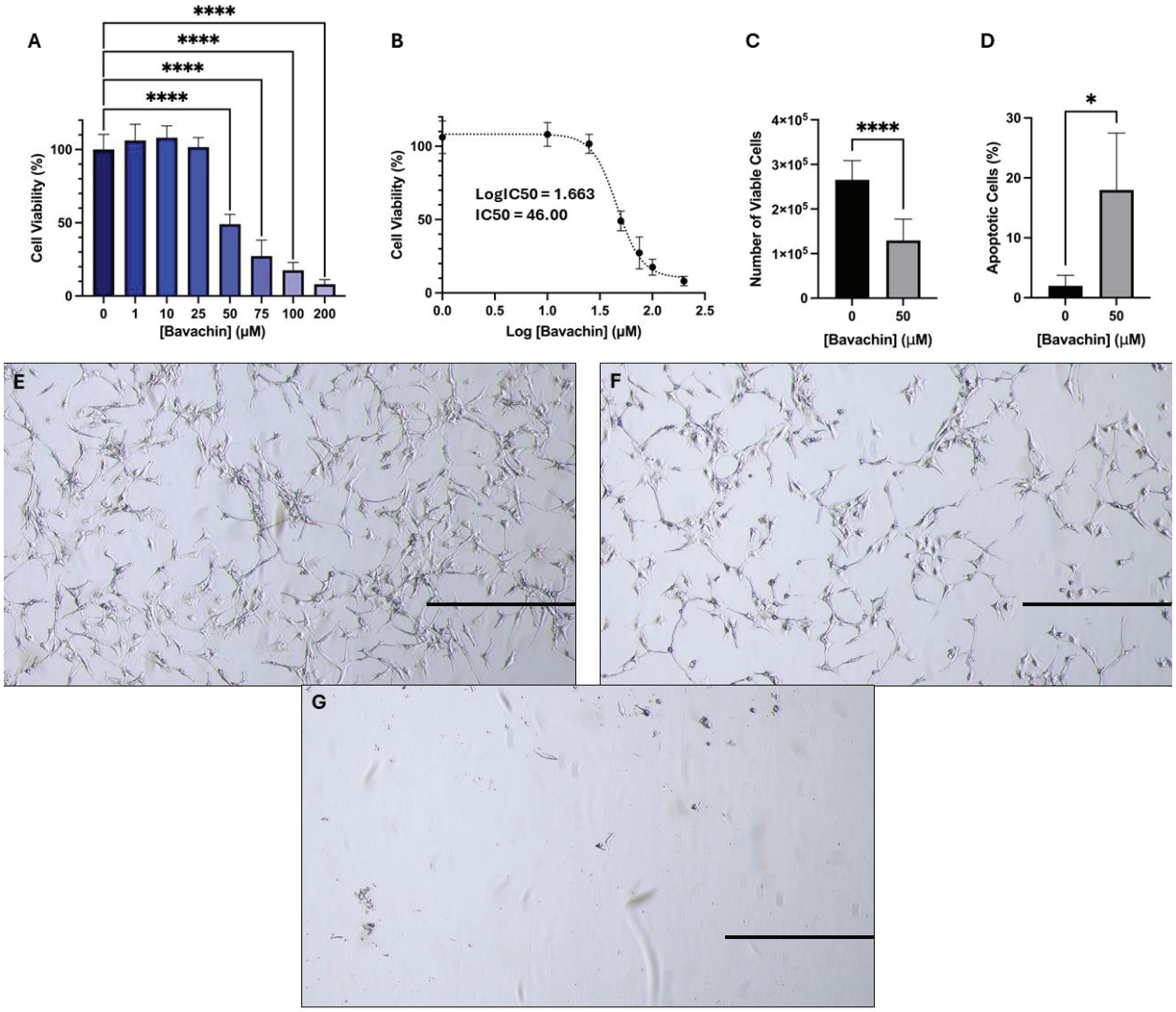

Figure 1. Cell viability after 48 hours of treatment with a range of bavachin concentrations. A. SRB staining following bavachin treatment in U-87 MG cells. B. Determination of the IC_{50} value from SRB assays. C. Trypan blue assay was conducted to determine the number of viable cells following bavachin treatment. D. Proportion of apoptotic cells determined from trypan blue assay. E-G. Phase contrast microscopy after bavachin treatment (0 μM (E), 50 μM (F), and 200 μM (G)). Scale bar = 0.5 mm. All images were taken at 40X total magnification.

Bavachin Inhibits the Growth of U-87 MG Stem Cells

Stem cells are a subset of cells within malignant neoplasms that drive tumor growth.[19] Tumorspheres, which are heterogeneous aggregates, typically contain hypoxic regions and cancer stem cells.[20] Compared to 2D cultures, 3D cell culture more effectively simulates the *in vivo* environment, as it allows for crucial cell-cell interactions and biological processes.[21] As a result, 3D cell culture is increasingly used in preclinical trials to more accurately assess therapeutic effects.[21] There was a significant reduction in tumorsphere volume after treatment with 50 μM and 100 μM of bavachin. Images of tumorspheres and the volume comparisons can be seen in **Figure 2**. **Figure 2A** and **Figure 2B** show no significant changes to tumorsphere size after treatment with 0 μM and 25 μM bavachin. **Figure 2C** and **Figure 2D** show tumorspheres of decreased volume after treatment with 50 μM and 100 μM bavachin, respectively. **Figure 2E** summarizes tumorsphere volume after treatment with different concentrations of bavachin and shows a significant decrease in tumorsphere volume in a concentration-dependent manner starting at 50 μM bavachin.

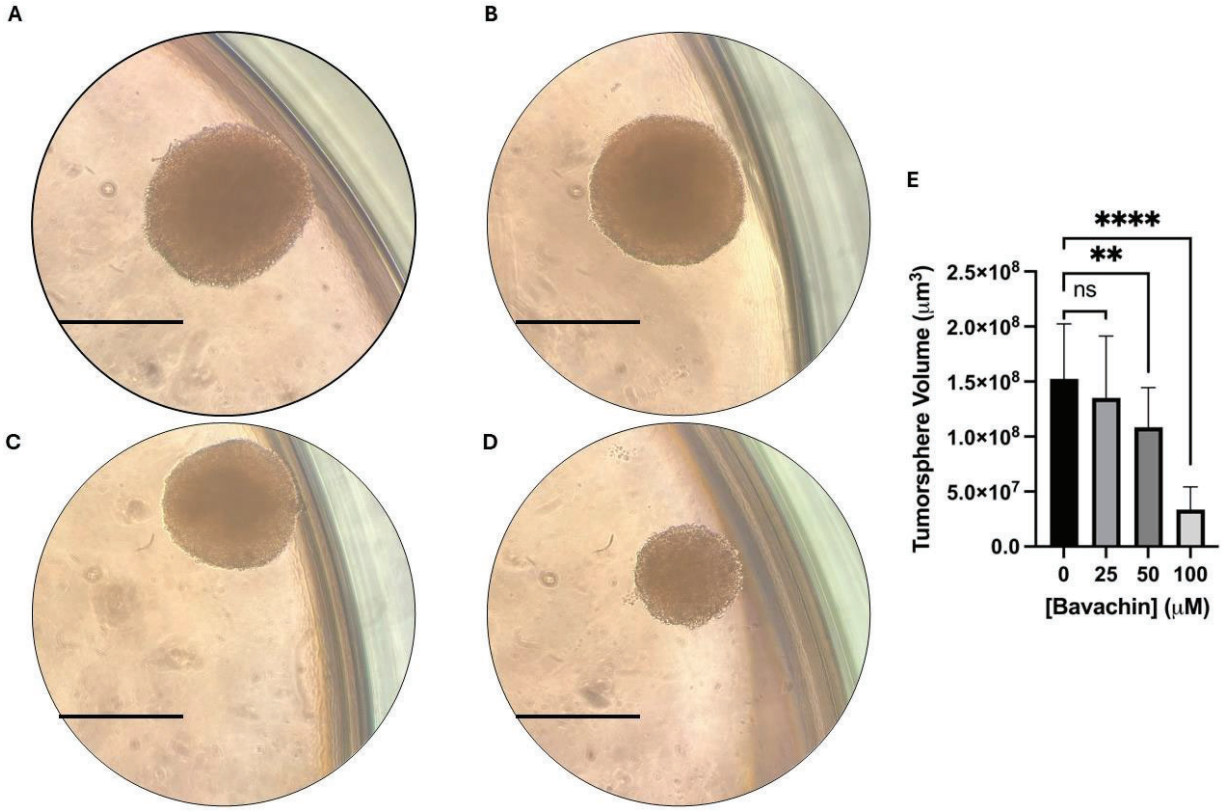

Figure 2. Bavachin inhibits U-87 MG stem cell growth. Images of tumorspheres after treatment with 0 μM (A), 25 μM (B), 50 μM (C), and 100 μM (D) of bavachin. All images were taken at 100X total magnification. Scale bar = 0.5 mm. Final tumorsphere volume was calculated for each treatment group (E).

Bavachin Exhibits Concentration-Dependent Interactions with Temozolomide

Bavachin and paclitaxel have reported to be synergistic in placental choriocarcinoma cells;[15] therefore, we were encouraged to test the effects of bavachin in combination with TMZ. A second set of SRB assays was performed using different combinations of bavachin and TMZ ranging from 10-50 μM bavachin and 500-1000 μM TMZ. **Figure 3A** shows cell viability after treatment with bavachin, TMZ, or a combination. Combination treatments using 50 μM bavachin with various concentrations of TMZ showed the largest decrease in cell viability. As seen in **Figure 3B**, combination treatments show an additive effect at 10 and 25 μM bavachin with TMZ, as well as 50 μM bavachin with 500 μM TMZ. An additive effect indicates that the combination of the two drugs is equal to the sum effects of the drugs administered independently. Seven out of the nine combinations of bavachin and TMZ tested demonstrated drug additivity. Interestingly, the combination of 50 μM bavachin and 750 μM TMZ exhibited drug antagonism, in which the combined effect fell in-between what was observed from the individual drug responses. The combination of 50 μM bavachin and 1000 μM TMZ exhibited significant drug synergy, in which the combined effect was greater than the sum of the individual effects.

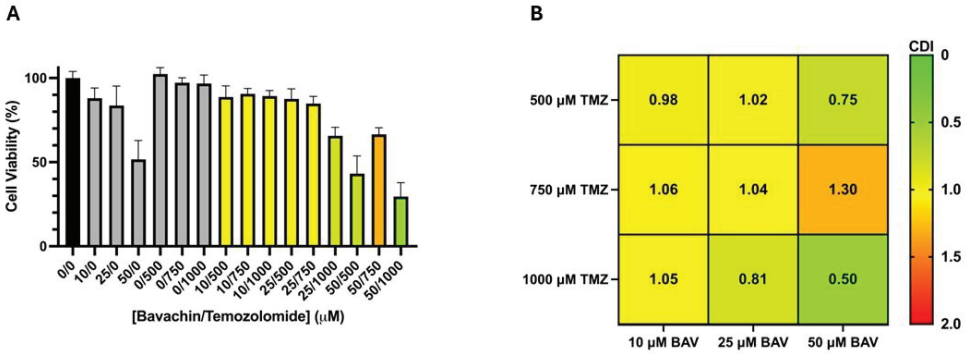

Figure 3. Cell viability after treatment with different concentrations of temozolomide, bavachin, or a combination. A. SRB assays were conducted to measure cell viabilities following 48 hours of the indicated treatment. B. Coefficient of drug interaction (CDI) values were calculated to determine drug additivity and synergy.

Bavachin Potentially Binds to Ornithine Decarboxylase to Inhibit U-87 MG Cell Growth

Although there have been several published findings on the effects of bavachin in cancer cells, [6, 15–18] a definitive binding target for bavachin has not yet been identified. We used ZINC20, CB-Dock2, and ChimeraX to determine and model the binding target of bavachin. **Figure 4A** shows a list of potential targets for bavachin from CB-Dock2. Human ornithine decarboxylase (ODC) was one of the most confident targets predicted by CB-Dock2 (p value = 1.558 X 10^{-39}). Since ODC expression has been implicated in glioblastomas,[28] we continued our analysis on ODC. Using the vina scores generated by CB-Dock2, it was determined that the best pocket for bavachin to bind on ODC is the C1 pocket. As seen in **Figure 4B**, the C1 pocket had the lowest Vina score of -9.2. The *in silico* model of bavachin and ODC using ChimeraX is shown in **Figure 4C**, and the predicted amino acid side chains of ODC that bind bavachin are identified in **Table 1**.

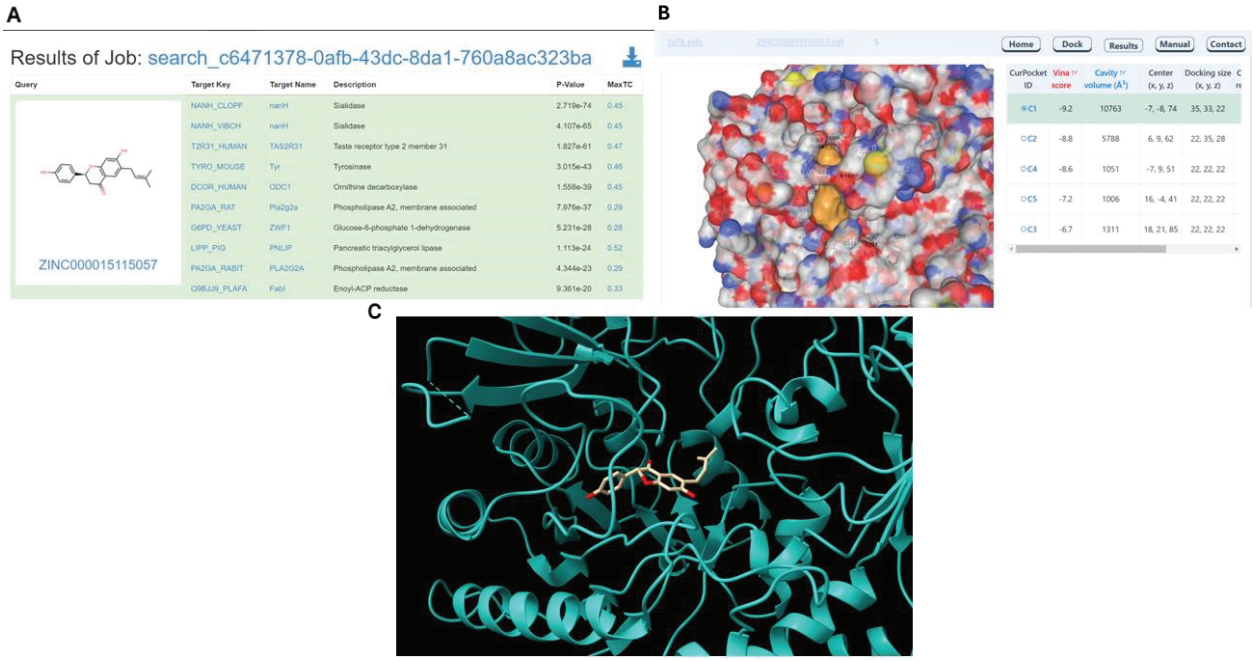

Figure 4. Bavachin potentially binds to ornithine decarboxylase**.** A. List of bavachin targets from CB-Dock2. B. Table of binding information for ODC from CB-Dock2. C. Image of bavachin (beige) binding to ODC (cyan) from ChimeraX.

Table 1. Predicted ornithine decarboxylase side chains in contact with bavachin. CB-Dock2 identified multiple amino acid residues in both chains of ODC that may bind bavachin. Highlighted residues are necessary for binding ornithine.[29]

Chain A	Chain B
TYR323 CYS360 ASP361 GLY362 LEU363 THR396 PHE397 ASN398 GLY399 GLN401	ALA67 VAL68 CYS70 ASP72 ASP88 CYS89 ALA90 THR93 GLU94 GLN96 LEU97 SER100 ARG154 ALA156 THR157 ASP158 ASP159 SER160 LYS161 ALA162 VAL168 LYS169 PHE170 HIS197 VAL198 GLY199 SER200 GLY201 GLY237 GLU274 GLY276 ARG277 TYR331 ASP332 HIS333 TYR389

DISCUSSION

Glioblastoma, a highly invasive brain tumor, often proves fatal despite surgical and chemotherapeutic interventions.[30] Temozolomide (TMZ), a common chemotherapy agent for glioblastoma (GBM), encounters resistance in GBM cells through anti-apoptotic, DNA repair, and drug efflux mechanisms.[3,4] This study aimed to evaluate the effects of bavachin, a natural compound known for inducing apoptosis in various cancer cells, in U-87 MG cells. Previous studies have shown that bavachin induces apoptosis by activating caspase-3 and caspase-9[6] and by reducing mitochondrial membrane potential.[15] Additionally, bavachin-induced apoptosis was associated with ER stress and the mitochondrial injury pathway (ROS/Mfn2/Akt) in HepG2 cells.[6] However, it is unknown if bavachin induces apoptosis in GBM cells via similar mechanisms.

After we determined an IC_{50} value of 46.00 μM for bavachin in U-87 MG cells, we proceeded with another assay designed to assess the apoptotic-inducing properties of bavachin: the trypan blue assay. Like the SRB assay, the trypan blue assay is used to determine cell viability. Our study provided evidence of bavachin's ability to induce apoptosis in GBM cells, with efficacy observed at 50 μM and higher concentrations. This study provided evidence that bavachin suppresses tumorsphere growth. These results are supported by a study in which bavachin suppressed placental choriocarcinoma tumorsphere growth.[15] However, the apparent IC_{50} of bavachin in our assay appeared higher than the IC_{50} of 46.00 μM calculated from the SRB assay. This finding

may reflect the challenges small molecules face in penetrating three-dimensional tumorspheres and equally affecting cell growth in a more heterogeneous cancer cell population.[31]

When used in combination, drugs can exhibit synergy, in which the effect of the drugs in combination is greater than the effect of either drug independently. Synergistic interactions are important in cancer treatment to enhance the effects of the treatment while reducing the adverse effects.[32] This is the reason many cancer patients are given "drug cocktails". This study revealed several combinations of bavachin and TMZ that resulted in drug additivity. While the combination of 50 μM bavachin and 750 μM TMZ resulted in drug antagonism, co-administration of 50 μM bavachin significantly enhanced the effects of TMZ at 1000 μM, indicating that bavachin may enhance the therapeutic efficacy of TMZ at these concentrations. These observed drug interaction patterns between bavachin and TMZ likely reflect their complex interactions *in vitro*. TMZ is an alkylating agent,[3] while we hypothesize bavachin inhibits ornithine decarboxylase (ODC) to reduce intracellular polyamine concentrations. At high (50/1000 μM) concentrations of both drugs, the combination may have overwhelmed the cells' DNA repair mechanisms; DNA damage from TMZ may have synergized with polyamine depletion caused by ODC inhibition, which has been shown to destabilize the double helix, leading to enhanced cell death.[33] However, at an intermediate concentration of TMZ (750 μM) and a high concentration of bavachin (50 μM), antagonism may have occurred because ODC inhibition still reduced polyamine levels, slowing cell proliferation. Since TMZ is most effective in rapidly dividing cells, its cytotoxic potential may be diminished under these conditions.[34] While the combination of bavachin and TMZ was assessed in an SRB assay, this combination was not tested in the tumorsphere assay. Future studies could expand upon these findings by investigating the potential synergistic effects of bavachin and TMZ in a more complex tumorsphere environment.

Molecular docking can be used to aid in drug discovery and development. Docking allows researchers to compare the binding of drugs to targets by creating *in silico* models. This study found that ODC is a potential target for bavachin. ODC represents an attractive target in GBM, as ODC activity is positively correlated with GBM pathology and negatively correlated with progression-free survival in GBM patients.[28] ODC is the first enzyme involved in polyamine synthesis.[35] Polyamines, including putrescine, spermidine, and spermine, are present in mammalian cells in millimolar concentrations. Polyamines are involved in cell growth by maintaining protein and nucleic acid synthesis, stabilizing chromatin structure, regulating apoptosis, and offering protection from oxidative damage. Polyamine metabolism is dysregulated in cancer indicating that increased levels of polyamines are necessary for cancer development and progression.[36] Glioblastoma multiforme is characterized by tumor-associated myeloid cells (TAMCs). ODC and polyamines were found to be upregulated in TAMCs.[37] We predict bavachin may inhibit ODC based on the binding score of ODC to bavachin and the involvement of ODC and polyamines in glioblastoma. Excitingly, bavachin is predicted to bind to ODC in the same active site as ornithine.[28] Inhibiting ODC in cancer cells would likely inhibit cancer growth and DNA repair.[32] Taken together, we can identify bavachin as a promising drug candidate for inhibiting GBM cell and tumorsphere growth.

CONCLUSIONS

In summary, our hypothesis was supported as bavachin reduced glioblastoma multiform cancer cell viability, induced apoptosis, inhibited tumorsphere growth, and enhanced temozolomide efficacy. Molecular docking analysis allowed us to identify ornithine decarboxylase as a possible target of bavachin, which should be evaluated *in vitro* in future studies.

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REFERENCES

1. American Cancer Society, Cancer, Key statistics for brain and spinal cord tumors, *https://www.cancer.org/cancer/types/brain-spinal-cordtumors-adults/about/key-statistics.html* (accessed Oct 2023)

2. van Rijn, J., Heimans, J. J., van den Berg, J., van der Valk, P. and Slotman, B. J. (2000) Survival of human glioma cells treated with various combinations of temozolomide and X-rays, *Int J Radiat Oncol Phys* 47, 779–784. *https://doi.org/10.1016/s0360-3016(99)00539-8*

3. Arora, A. and Somasundaram, K. (2019) Glioblastoma vs temozolomide: can the red queen race be won?, *Cancer Biol Ther* 20, 1083–1090. *https://doi.org/10.1080/15384047.2019.1599662*

4. Singh, N., Miner, A., Hennis, L., and Mittal, S. (2021) Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review, *Cancer Drug Resist* 4, 17–43. *https://doi.org/10.20517/cdr.2020.79*

5. Mayo Clinic, Glioblastoma, https://www.mayoclinic.org/diseases-conditions/glioblastoma/cdc-20350148 (accessed Oct 2023)

6. Yang, Y., Tang, X., Hao, F., Ma, Z., Wang, Y., Wang, L., and Gao, Y. (2018) Bavachin induces apoptosis through mitochondrial regulated ER stress pathway in HepG2 cells, *Biol Pharm Bull* 41, 198–207. *https://doi.org/10.1248/bpb.b17-00672*

7. Abotaleb, M., Samuel, S. M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., and Büsselberg, D. (2018) Flavonoids in cancer and apoptosis, *Cancers* 11(1), 28. *https://doi.org/10.3390/cancers11010028*

8. D'Archivio, M., Santangelo, C., Scazzocchio, B., Varì, R., Filesi, C., Masella, R., and Giovannini, C. (2008) Modulatory effects of polyphenols on apoptosis induction: relevance for cancer prevention, *International Journal of Molecular Sciences* 9(3), 213–228. *https://doi.org/10.3390/ijms9030213*

9. Giordano, A., and Tommonaro, G. (2019) Curcumin and cancer, *Nutrients* 11(10), 2376. *https://doi.org/10.3390/nu11102376*

10. Zhang, D. C., Liu, J. L., Ding, Y. B., Xia, J. G., and Chen, G. Y. (2013) Icariin potentiates the antitumor activity of gemcitabine in gallbladder cancer by suppressing NF-κB, *Acta Pharmacologica Sinica* 34(2), 301–308. *https://doi.org/10.1038/aps.2012.162*

11. Dixon, R. A. and Pasinetti, G. M. (2010) Flavonoids and isoflavonoids: from plant biology to agriculture and neuroscience, *Plant Physiol* 154, 453–457. *https://doi.org/10.1104/pp.110.161430*

12. Havsteen, B. H. (2002) The biochemistry and medical significance of the flavonoids, *Pharmacol Ther* 96, 67–202. *https://doi.org/10.1016/s0163-7258(02)00298-x*

13. Peterson, J. J., Dwyer, J. T., Jacques, P. F. and McCullough, M. L. (2012) Associations between flavonoids and cardiovascular disease incidence or mortality in European and US populations, *Nutr Rev* 70, 491–508. *https://doi.org/10.1111/j.1753-4887.2012.00508.x*

14. Chen, L., Chen, S., Sun, P., Liu, X., Zhan, Z. and Wang, J. (2023) Psoralea corylifolia L.: a comprehensive review of its botany, traditional uses, phytochemistry, pharmacology, toxicology, quality control and pharmacokinetics, *Chin Med* 18, 4. *https://doi.org/10.1186/s13020-022-00704-6*

15. Lee, J., Lim, W. and Song, G. (2020) Bavachin suppresses human placental choriocarcinoma cells by targeting electron transport chain complexes and mitochondrial dysfunction, *Free Radical Biol Med* 156, 26–35. *https://doi.org/10.1016/j.freeradbiomed.2020.05.022*

16. Luo, Y., Gao, X., Zou, L., Lei, M., Feng, J. and Hu, Z. (2021) Bavachin induces ferroptosis through the STAT3/P53/SLC7A11 axis in osteosarcoma cells, *Oxid Med Cell Longev* 2021, 1–14. *https://doi.org/10.1155/2021/1783485*

17. Takeda, T., Tsubaki, M., Tomonari, Y., Kawashima, K., Itoh, T., Imano, M., Satou, T., and Nishida, S. (2018) Bavachin induces the apoptosis of multiple myeloma cell lines by inhibiting the activation of nuclear factor kappa B and signal transducer and activator of transcription 3, *Biomed Pharmacother* 100, 486–494. *https://doi.org/10.1016/j.biopha.2018.02.019*

18. Wang, M., Tian, B., Shen, J., Xu, S., Liu, C., Guan, L., Guo, M., and Dou, J. (2023) Bavachin induces apoptosis in colorectal cancer cells through Gadd45a via the MAPK signaling pathway, *Chin J Nat Med* 21, 36–46. *https://doi.org/10.1016/S1875-5364(23)60383-8*

19. Davidson, C. D., Tomczak, J. A., Amiel, E., Carr, F. E. (2022) Inhibition of glycogen metabolism induces reactive oxygen species-dependent cytotoxicity in anaplastic thyroid cancer in female mice, *Endocrinology* 163(12). *https://doi.org/10.1210/endocr/bqac169*

20. Hong, X., Chedid, K. and Kalkanis, S. N. (2012) Glioblastoma cell line-derived spheres in serum-containing medium versus serum-free medium: A comparison of cancer stem cell properties, *Int J Oncol* 41, 1693–1700. *https://doi.org/10.3892/ijo.2012.1592*

21. Vinci, M., Gowan, S., Boxall, F., Patterson, L., Zimmermann, M., Court, W., Lomas, C., Mendiola, M., Hardisson, D., and Eccles, S. A. (2012) Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation, *BMC Biol* 10, 29–29. *https://doi.org/10.1186/1741-7007-10-29*

22. Wanigasekara, J., Carroll, L. J., Cullen, P. J., Tiwari, B. and Curtin, J. F. (2023) Three-dimensional (3D) in vitro cell culture protocols to enhance glioblastoma research, *PloS One,* 18(2) e0276248. *https://doi.org/10.1371/journal.pone.0276248*

23. Irwin, J. J., Tang, K. G., Young, J., Dandarchuluun, C., Wong, B. R., Khurelbaatar, M., Moroz, Y. S., Mayfield, J., and Sayle, R. A. (2020) ZINC20-A free ultralarge-scale chemical database for ligand discovery, *J Chem Inf Model* 60, 6065–6073. *https://doi.org/10.1021/acs.jcim.0c00675*

24. Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z. and Cao, Y. (2022) CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting, *Nucleic Acids Res* 50, W159–W164. *https://doi.org/10.1093/nar/gkac394*

25. Yang, X., Liu, Y., Gan, J., Xiao, Z. and Cao, Y. (2022) FitDock: protein–ligand docking by template fitting, *Brief Bioinform* 23(3). *https://doi.org/10.1093/bib/bbac087*

26. Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., and Ferrin, T. E. (2023) UCSF ChimeraX: Tools for structure building and analysis, *Protein Sci* 32(11) e4792. *https://doi.org/10.1002/pro.4792*

27. Hao, J., Li, Q., Xu, S., Shen, Y., and Sun, G. (2008) Effect of lumiracoxib on proliferation and apoptosis of human nonsmall cell lung cancer cells in vitro, *Chin Med J (Engl)* 121, 602–607.
28. Levin, V. A., Jochec, J. L., Shantz, L. M., and Aldape, K. D. (2007). Relationship between ornithine decarboxylase levels in anaplastic gliomas and progression-free survival in patients treated with DFMO–PCV chemotherapy, *Int J Cancer* 121, 2279–2283. *https://doi.org/10.1002/ijc.22914*
29. Oliveira, E. F., Cerqueira, N. M., Fernandes, P. A., and Ramos, M. J. (2011). Mechanism of formation of the internal aldimine in pyridoxal 5'-phosphate-dependent enzymes, *J Am Chem Soc*, 133(39), 15496–15505. *https://doi.org/10.1021/ja204229m*
30. American Cancer Society, Cancer, What are Adult Brain and Spinal Cord Tumors? *https://www.cancer.org/cancer/types/brain-spinal-cord-tumors-adults/about/what-are-brain-spinal-tumors.html* (accessed Oct 2023)
31. Kim, S., and Alexander, C. M. (2014) Tumorsphere assay provides more accurate prediction of in vivo responses to chemotherapeutics, *Biotechnology Letters* 36(3), 481–488. *https://doi.org/10.1007/s10529-013-1393-1*
32. Roell, K. R., Reif, D. M., and Motsinger-Reif, A. A. (2017) An introduction to terminology and methodology of chemical synergy—perspectives from across disciplines, *Front Pharmacol* 8, 158. *https://doi.org/10.3389/fphar.2017.00158*
33. Zhao, C., Ma, Y., Zhang, M., Gao, X., Liang, W., Qin, Y., Fu, Y., Jia, M., Song, H., Gao, C., and Zhao, W. (2023) Polyamine metabolism controls B-to-Z DNA transition to orchestrate DNA sensor cGAS activity, *Immunity* 56(11), 2508–2522.e6. *https://doi.org/10.1016/j.immuni.2023.09.012*
34. Moiseeva, N. I., Susova, O. Y., Mitrofanov, A. A., Panteleev, D. Y., Pavlova, G. V., Pustogarov, N. A., Stavrovskaya, A. A., and Rybalkina, E. Y. (2016) Connection between proliferation rate and temozolomide sensitivity of primary glioblastoma cell culture and expression of YB-1 and LRP/MVP, *Biochemistry (mosc)* 81(6), 628–635. *https://doi.org/10.1134/S0006297916060109*
35. Gerner, E. W. and Meyskens, F. L., Jr (2004) Polyamines and cancer: old molecules, new understanding, *Nat Rev Cancer* 4(10), 781–792. *https://doi.org/10.1038/nrc1454*
36. Casero, J., Robert, A., Murray S. T., Pegg, A. E. (2018) Polyamine metabolism and cancer: treatments, challenges and opportunities, *Nat Rev Cancer* 18, 681–695. *https://doi.org/10.1038/s41568-018-0050-3*
37. Miska, J., Rashidi, A., Lee-Chang, C., Gao, P., Lopez-Rosas, A., Zhang, P., Burga, R., Castro, B., Xiao, T., Han, Y., Hou, D., Sampat, S., Cordero, A., Stoolman, J. S., Horbinski, C. M., Burns, M., Reshetnyak, Y. K., Chandel, N. S., and Lesniak, M. S. (2021) Polyamines drive myeloid cell survival by buffering intracellular pH to promote immunosuppression in glioblastoma, *Sci Adv* 7(8), eabc8929. *https://doi.org/10.1126/sciadv.abc8929*

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PRESS SUMMARY

Glioblastoma multiforme is one of the most aggressive forms of cancer with few effective treatments. The current first-line treatment temozolomide results in harmful side effects and tumor recurrence. Bavachin is a flavonoid that has been tested in multiple cancer cell lines including oral, bone, multiple myeloma, choriocarcinoma, colorectal, and liver cancer but not glioblastoma. This study determined that bavachin is effective at killing glioblastoma cells and can enhance the effects of temozolomide.