ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

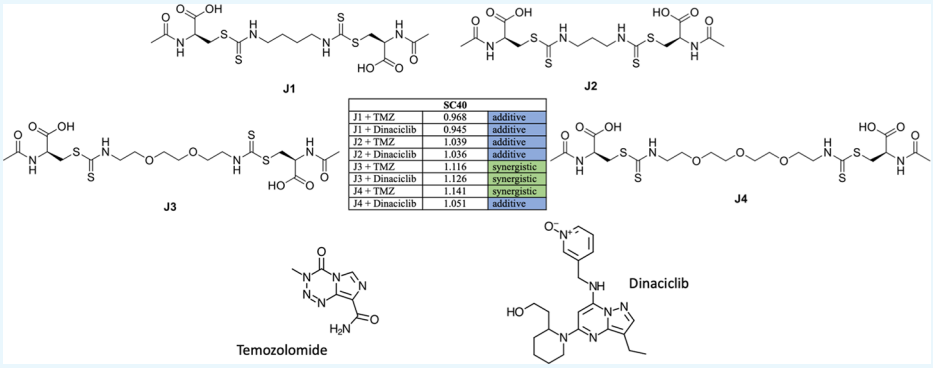
Diisothiocyanate-Derived Mercapturic Acids Are a Promising Partner for Combination Therapies in Glioblastoma

Pengfei Xu, Mike-Andrew Westhoff, Amina Hadzalic, Klaus-Michael Debatin, Lukasz Winiarski, Jozef Oleksyszyn, Christian Rainer Wirtz, Uwe Knippschild, and Timo Burster*

Cite This: *ACS Omega* 2022, 7, 5929−5936

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Glioblastoma represents the most aggressive tumor of the central nervous system. Due to invasion of glioblastoma stem cells into the healthy tissue, chemoresistance, and recurrence of the tumor, it is difficult to successfully treat glioblastoma patients, which is demonstrated by the low life expectancy of patients after standard therapy treatment. Recently, we found that diisothiocyanate-derived mercapturic acids, which are isothiocyanate derivatives from plants of the Cruciferae family, provoked a decrease in glioblastoma cell viability. These findings were extended by combining diisothiocyanate-derived mercapturic acids with dinaciclib (a small-molecule inhibitor of cyclin-dependent kinases with anti-proliferative capacity) or temozolomide (TMZ, standard chemotherapeutic agent) to test whether the components have a cytotoxic effect on glioblastoma cells when the dosage is low. Here, we demonstrate that the combination of diisothiocyanate-derived mercapturic acids with dinaciclib or TMZ had an additive or even synergistic effect in the restriction of cell growth dependent on the combination of the components and the glioblastoma cell source. This strategy could be applied to inhibit glioblastoma cell growth as a therapeutic interference of glioblastoma.

1. INTRODUCTION

Glioblastoma (astrocytoma grade IV) is the most aggressive tumor of the central nervous system. Glioblastoma cells are highly mobile and invasive, easily repopulating the tumor bulk after surgical resection. Additionally, glioblastoma stem cells are considered to be particularly chemoresistant, making a complete therapeutic elimination of these cells almost impossible.[1] After surgery, radiation, and chemotherapy with temozolomide (TMZ), a standard chemotherapeutic agent used to treat glioblastoma, the survival period for patients diagnosed with glioblastoma remains low, between twelve to fifteen months,[2,3] indicating the need for novel and effective reagents that can be applied in combination with existing chemotherapeutics to overcome resistant mechanisms.

Dinaciclib is a small-molecule inhibitor of cyclin-dependent kinases (CDKs). CDKs contribute to tumor cell progression, and dinaciclib has been shown to exert anti-proliferative effects by inhibiting several CDKs including CDK9.[4] In contrast to dinaciclib, isothiocyanates (ITCs) are natural products of the Cruciferae family of plants, such as broccoli or radish, and interfere with tumor cell growth.[5,6] ITCs selectively cause an accumulation of reactive oxygen species (ROS), which in turn provoke apoptosis in tumor cells. Prominently, healthy, non-transformed cells are less susceptible to ROS.[7] Previous studies have reported the synthesis of diisothiocyanate-derived mercapturic acids which were able to kill human adenocarcinoma cells.[8] In this context, we found that the application of diisothiocyanate-derived mercapturic acids (J1, J2, J3, and J4) interfered with cell viability of glioblastoma cells and glioblastoma stem cells.[9] Here, we extend our examination by combining diisothiocyanate-derived mercapturic acids with

Received: November 3, 2021
Accepted: December 24, 2021
Published: February 8, 2022

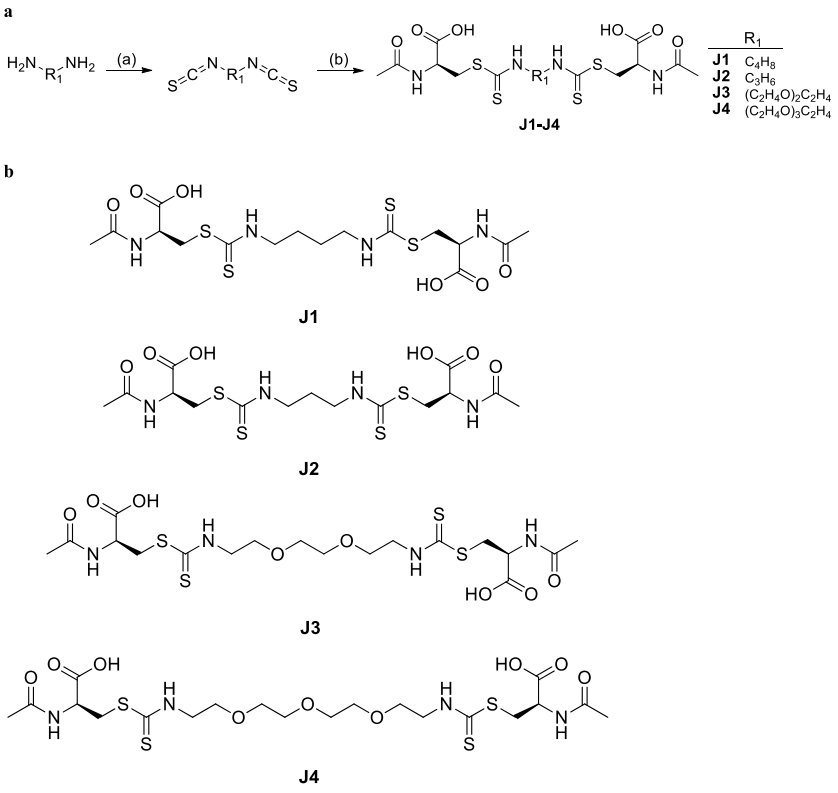


Figure 1. Scheme of diisothiocyanate-derived mercapturic acids. (a) General method for diisothiocyanate-derived mercapturic acids synthesis. (a)—(1) CS_2, Et_3N, DMF, 0 °C, 15 min; (2) HBTU, 0 °C, 15 min; (b)—*N*-acetyl-L-cysteine, $NaHCO_3$, H_2O, isopropanol, RT. (b) Chemical structures of diisothiocyanate-derived mercapturic acids: J1, J2, J3, and J4.[8]

dinaciclib or TMZ and present that the combination of diisothiocyanate-derived mercapturic acids with dinaciclib or TMZ supplement their effect to reduce cell viability, including primary glioblastoma cells from a glioblastoma patient.

2. RESULTS AND DISCUSSION

2.1. Diisothiocyanate-Derived Mercapturic Acids Combined with Dinaciclib or TMZ Reduce the Metabolic Activity in Glioblastoma Cell Line U87. In a first set of experiments, the EC_{50} (half-maximal effective concentration) values were precisely determined for diisothiocyanate-derived mercapturic acid compounds (J1, J2, J3, and J4, Figure 1), dinaciclib, and TMZ by titrating different concentrations of these compounds to the established glioblastoma cell line U87, sphere-cultured stem cell-enriched glioblastoma cell populations (SCs) that were generated from the tissue of a patient diagnosed with glioblastoma (SC40), and primary differentiated glioblastoma cells (SCs adhesively cultured in the presence of FBS, PC40, Supporting Information S1). The metabolic activity was analyzed by MTT assay and expressed as a dose response curve. Having verified the precise EC_{50}, glioblastoma cell line U87 was incubated with compound J1 (0.17 μM), J2 (0.25 μM), J3 (1.1 μM), J4 (0.65 μM), dinaciclib (7 nM), TMZ (21.5 μM), or a combination of J1, J2, J3, or J4 with dinaciclib or TMZ and cell viability was analyzed. J1, J2, J3, J4, dinaciclib, and TMZ significantly reduced U87 cell viability and cell density (Figures 2 and 3). The combination of J1 with dinaciclib or TMZ significantly decreased further metabolic activity of U87; this was more prominent when using the combination of J1 and dinaciclib. In addition, the combination of J2 or J3 with dinaciclib reduced

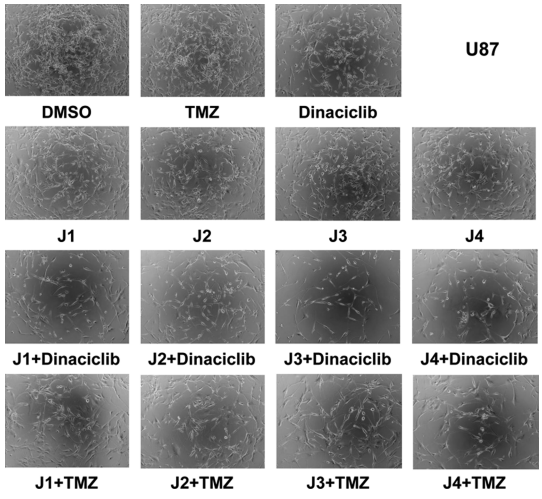


Figure 2. U87 cell density was analyzed by light microscopy. U87 cells were treated with EC_{50} of J1, J2, J3, J4, dinaciclib, or TMZ or a combination of J1, J2, J3, or J4 with dinaciclib or TMZ. DMSO served as a vehicle control. After an incubation time of three days, the medium was removed and cells were treated with the respective components for three additional days. Magnification of microscopic images was 10×.

cell viability but the combination of J2 or J3 with TMZ and J4 with dinaciclib or TMZ did not reach statistical significance.

2.2. Diisothiocyanate-Derived Mercapturic Acids Combined with Dinaciclib or TMZ Reduce the Metabolic Activity in Primary Glioblastoma Cells. In order to test the components on patient-derived glioblastoma stem cells and primary glioblastoma cells, SC40 were treated with J1 (2.6

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

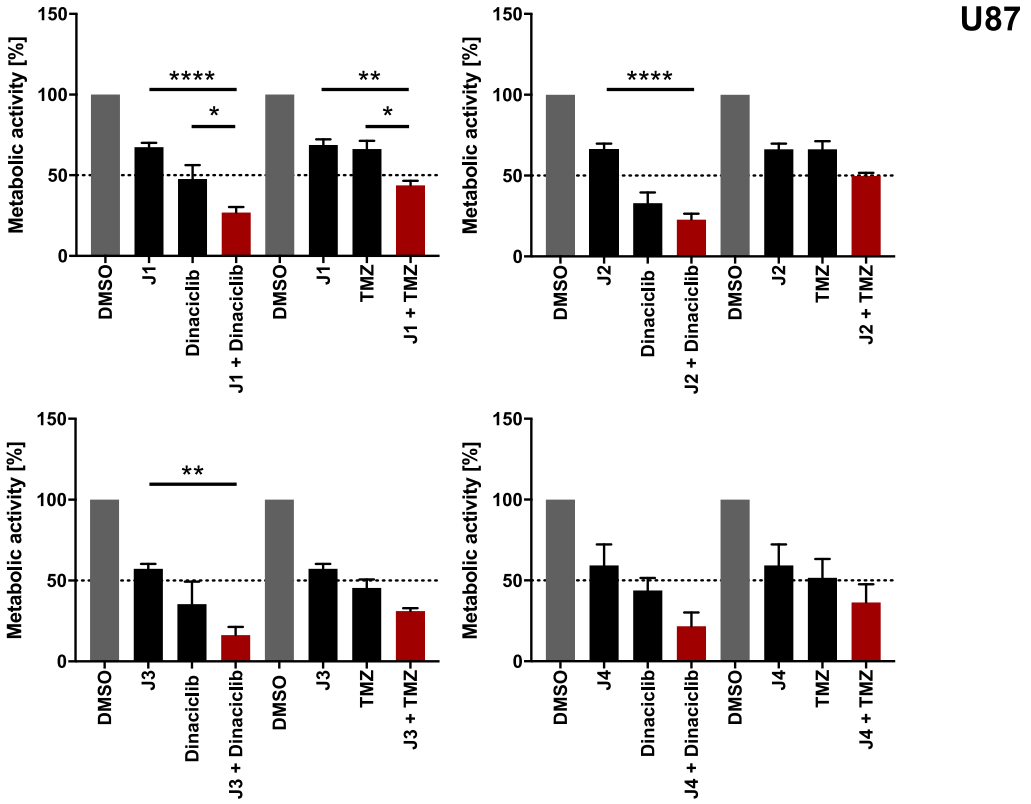


Figure 3. Metabolic activity in U87 was determined by MTT assay. The metabolic activity in human glioblastoma cell line U87 was determined by MTT assay. U87 cells were cultured with J1, J2, J3, J4, dinaciclib, or TMZ or J1, J2, J3, or J4 in combination with dinaciclib or TMZ. Cells were incubated for three days, the medium was removed, the cells were treated with the respective components for three additional days, and cell viability was measured. The DMSO sample served as a vehicle control. The MTT assay was performed in triplicate and with three independent experiments ($n = 3$). TMZ, temozolomide; EC_{50}, drug concentration yielding half-maximal response. Only significance between the components is shown. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), or $P < 0.0001$ (****).

μM), J2 (1.8 μM), J3 (2.4 μM), J4 (1.95 μM), dinaciclib (9 nM), or TMZ (14.01 μM) and PC40 incubated with J1 (0.2 μM), J2 (0.2 μM), J3 (0.78 μM), J4 (0.5 μM), dinaciclib (8 nM), TMZ (15.76 μM), or a combination of J1, J2, J3, or J4 with dinaciclib or TMZ. SC40 and PC40 cells were susceptible to diisothiocyanate-derived mercapturic acids, dinaciclib, and TMZ (Figures 4 and 5). The combination of J3 or J4 with dinaciclib or TMZ decreased SC40 cell viability significantly (Figure 4). In the case of PC 40, the combination of J2 or J4 with dinaciclib or TMZ significantly decreased cell viability (Figure 5). Primary astrocyte-enriched cultures, which was used as control cells, treated with J1, J2, J3, or J4 did not provoke any changes in metabolic activity; only the combination of J3 with TMZ reduced the cell viability in primary astrocytes (Figure 6). Next, TMZ-resistant glioblastoma T98G cells were incubated with compounds J1 (0.17 μM), J2 (0.25 μM), J4 (0.65 μM), and TMZ (50 μM). We observed that the diisothiocyanate-derived mercapturic acid could not sensitize T98 for TMZ, but a higher amount of these components (excess) had a strong effect on cell viability (Supporting Information S2).

While the combination of J2 with TMZ showed an antagonistic outcome, J1, J2, J3, or J4 with dinaciclib or J1, J3, or J4 with TMZ have an additive effect, when analyzing the U87 data by Bliss analysis (Table 1). Moreover, SC40 and PC40 showed additive and synergistic results (comparable to published data treating PC with imipridones, a class of small molecules used for anti-seizure medication, in combination with 2-deoxyglucose, showed a synergistic inhibitory effect to PC[10]), whereby additional synergy between combinations of J3 with TMZ, J3 with dinaciclib, and J4 with TMZ were found for SC40. Similar results were obtained from PC40, where synergistic effects were due to the combination of J3 with dinaciclib, J4 with TMZ, and J4 with dinaciclib.

Recently, we demonstrated that J1, J2, J3, and J4[8] were selectively cytotoxic to glioblastoma cells.[9] However, SC40 treated with J1, J2, J3, or J4 (at EC_{50}) together with TMZ (3.09 μM), a concentration found in the brain of patients after being therapeutically treated with TMZ,[11] had neither a stabilizing nor a sensitizing effect on SC40. One reason might be that glioblastoma cells were treated with component J1, J2, J3, or J4 in combination with TMZ for a limited time (three days). This latter aspect is of particular importance, as these glioblastoma stem cells proliferate slowly[12] and the current model of how TMZ affects cells postulates several rounds of cell cycle progression and futile repair attempts.[13] Here, we increased the time of treatment up to seven days. Indeed, J1, J2, J3, or J4 combined with dinaciclib or TMZ showed additive or even synergistic results on the reduction of cell viability depending on the source of glioblastoma cells, in contrast to when compounds were used separately.

The current standard treatment for glioblastoma patients encompasses a combination of radiotherapy and chemotherapy after surgery; however, most clinical trials of diverse molecular targeted therapies for glioblastoma have not revealed a significant survival advantage.[14] Thus, novel chemotherapy

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

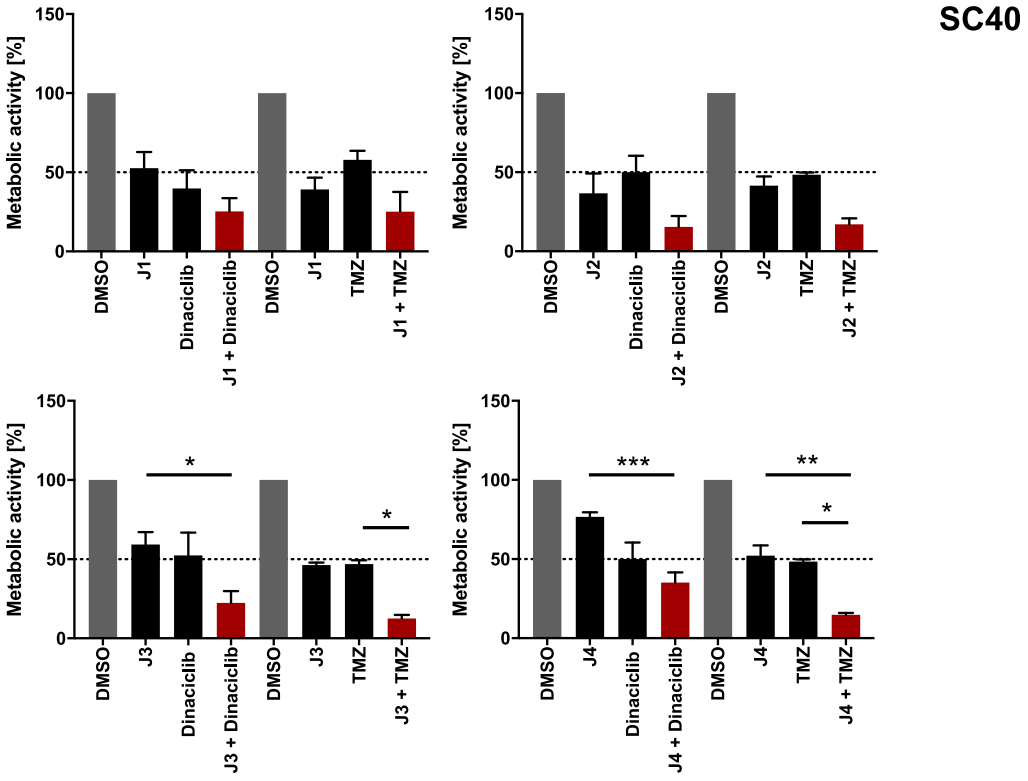


Figure 4. Determination of cell viability of SC40. Sphere-cultured stem cell-enriched glioblastoma cell population from patient 40 (SC40) was incubated with J1, J2, J3, J4, dinaciclib, or TMZ or J1, J2, J3, or J4 in combination with dinaciclib or TMZ, after an incubation time of three days the medium was removed and SC40 cells were treated with the respective components (three days). The metabolic activity was determined by using an MTT assay. Three independent experiments were performed (n = 3).

strategies are needed in combination with multi-targeted drugs and immunotherapy in addition to surgery and radiotherapy in order to overcome tumor resistance.[14,15] The most commonly used chemotherapeutic drug, TMZ, methylates DNA leading to double-stranded DNA breaks and eventually to apoptosis.[16] In general, dinaciclib limits glioma cell growth by inhibiting CDKs, promotes degradation of Mcl-1 by the proteasome and enhances ABT-737-mediated cell death of glioma cell lines.[17,18] ITC can selectively trigger the accumulation of ROS, leading to apoptosis of transformed cells.[7]

3. CONCLUSIONS

Combinatorics, which provokes double-stranded DNA breaks (TMZ) or the CDKs inhibitor (dinaciclib) with ROS-induced components (ITC or diisothiocyanate-derived mercapturic acids), might be advantageous in targeting different pathways for therapeutic treatment of glioblastoma.

4. METHODS

4.1. Diisothiocyanate-Derived Mercapturic Acids. Diisothiocyanate-derived mercapturic acids were generated as described.[8] Briefly, J3 and J4 diisothiocyanates were synthesized from diamine (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) by using carbon disulfide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and triethylamine (Avantor Performance Materials Poland S.A., Gliwice, Poland) for generation in situ ditiocarbamate salt and followed by an addition of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (Iris Biotech GmbH, Marktredwitz, Germany) as a desulfurization agent. The diisothiocyanates for J1 and J2 were purchased from Sigma-Aldrich (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). To obtain diisothiocyanate-derived mercapturic acid, diisothiocyanate (dissolved in isopropanol) was added to an aqueous solution of *N*-acetyl-L-cysteine (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and sodium hydrogen carbonate (Avantor Performance Materials Poland S.A.). The product was purified by HPLC (Discovery BIO Wide Pore C8; 10 μm, 25 cm × 21.2 mm).

4.2. Cells. The human glioblastoma cell line U87-MG (U87, American Type Culture Collection, ATCC, Manassas, VA, USA) was grown in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc.) with 10% FBS and 1% penicillin (120 mg/mL) combined with streptomycin (120 mg/mL) at 37 °C (5% CO_2 atmosphere).

Sphere-cultured stem cell-enriched glioblastoma cell populations (SCs) were generated from the tissue of a patient diagnosed with astrocytoma grade IV (glioblastoma, patient number 40, 57 years, female, SC40),[12] which was minced, washed in PBS, and incubated with TrypLE Express (Gibco; Thermo Fisher Scientific, Inc.). Afterward, cells were filtered, cultured at 37 °C (5% CO_2 atmosphere) in DMEM/Ham's F-12 medium (Gibco; Thermo Fisher Scientific, Inc.) containing L-glutamine, 0.01% (v/v) epidermal growth factor (EGF; Biomol GmbH, Hamburg, Germany), 0.04% (v/v) fibroblast growth factor (FGF; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), 1% (v/v) B27 (Gibco; Thermo Fisher Scientific, Inc.), 2% fungizone (Gibco; Thermo Fisher Scientific, Inc.), and 1% penicillin (120 mg/mL)/streptomycin (120 mg/mL; Thermo Fisher Scientific, Inc.),[12] and defined as sphere-cultured stem cell-enriched glioblastoma cell populations according to the patient number from which they were derived (SC40), expressing stem cell and differentiation

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

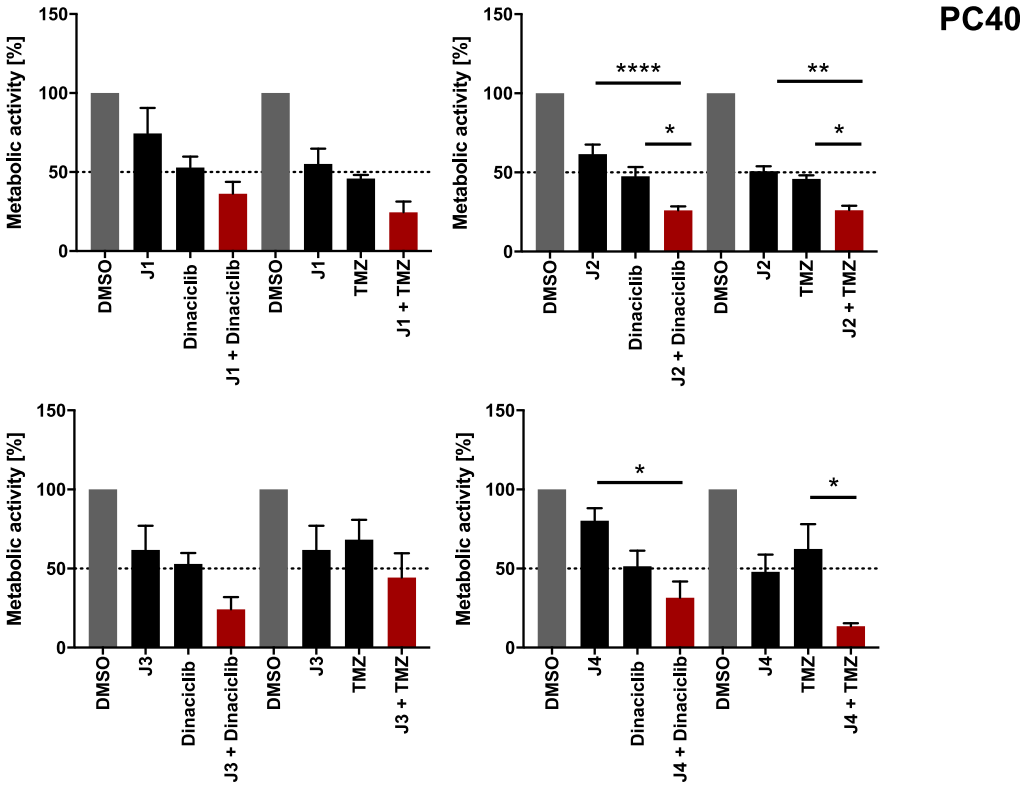


Figure 5. Determination of cell viability of PC40. SC40-derived primary differentiated cells (PC40) were co-cultured with J1, J2, J3, J4, dinaciclib, or TMZ or J1, J2, J3, or J4 in combination with dinaciclib or TMZ for three days. Afterward, the medium was removed and PC40 cells were treated with the components for three days. The cell viability was measured by using an MTT assay. Three independent experiments were performed (n = 3).

markers.[19] PC40 cells are adherent glioblastoma cells which were generated from SC40.[12] SC40 were kept at 37 °C (5% CO_2 atmosphere) in DMEM supplemented with 10% FBS plus 2 mM glutamine, 1% penicillin (120 mg/mL)/streptomycin (120 mg/mL; Thermo Fisher Scientific, Inc.) since SCs differentiated into PCs when FBS was added.

Primary astrocyte-enriched cultures, which are patient-derived non-tumor cell culture isolated and grown from surgical specimen after patient's consent was obtained as described previously,[20] were treated according to the protocol for human astrocytes (Thermo Fisher Scientic Inc.). Cells were maintained in Dulbecco's Modified Eagle Medium with high glucose (DMEM+ GlutaMax; Gibco, Thermo Fisher Scientic Inc.), and supplemented with 10% fetal calf serum (Gibco), 1% N-2 (Gibco), 0.01% human EGF (Biomol), and 1% penicillin/streptomycin (Gibco). Cell culture plates were coated with Geltrex matrix (reduced growth factor basement membrane matrix) (Gibco) for 1 h prior to cell seeding. Plates were then washed with DPBS + Ca/Mg (Gibco), and cells were seeded at a cell density of 1×10^4 cells/cm^2. After overnight incubation, cells were treated with J1, J2, J3 J4, TMZ, dimethyl sulfoxide (DMSO), or the combination. Following an incubation time of 3 days, the media were removed and cells were again treated with the respective components. The metabolic activity (MTT test) was assessed after 3 additional days.

The use of SCs and primary astrocyte-enriched cultures for experiments was approved by the local ethics committee of Ulm University, Germany, number: 162/10.

4.3. Determination of Cellular Metabolic Activity and Cell Viability by Using MTT Test. Adherent glioblastoma cells (U87, PC40, or T98G from the ATCC) were placed in 96-well flat-bottomed tissue culture plates at 1.5×10^4 cells/mL in 100 μL DMEM containing 10% FBS and 1% penicillin/streptomycin. SC40 were seeded in 96-well flat-bottomed tissue culture plates at 1.5×10^4 cells/mL in 90 μl DMEM/Ham's F-12 containing 0.01% EGF, 0.04% FGF, 1% B27, 2% fungizone, and 1% penicillin/streptomycin. After an incubation time of one day, the medium was removed. Different concentrations of J1, J2, J3, J4, dinaciclib (ChemieTek, Indianapolis, IN, USA), TMZ (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), or the solvent and vehicle control DMSO (Serva Electrophoresis GmbH, Heidelberg, Germany) were prepared in DMEM containing 1.5% FBS and 1% penicillin/streptomycin and added to U87 and PC40 cells (final volume, 100 μL). In the case of SC40, the medium was not changed and J1, J2, J3, J4, dinaciclib, TMZ, or DMSO were added directly (final volume, 100 μL) and cells were cultured for three days. The medium was removed, and J1, J2, J3, J4, dinaciclib, TMZ, or DMSO was added and incubated for an additional three days (in total, cells were cultured for seven days). The medium was removed, and U87 and PC40 cells were incubated with 100 μL MTT working solution (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), diluted 1:5 in RPMI 1640 medium without L-glutamine and phenol red. For SC40, the SC40 plate was centrifuged for 390g for 5 min at room temperature. The supernatant was then discarded, the cells re-suspended in 100 μL MTT working solution, and incubated for an additional 3 h at 37 °C. Formazan crystals were solubilized with 100 μL isopropyl alcohol, and cell viability was tested by optical density at 550 nm using a microplate spectrometer (Tecan Spectra Classic, Tecan Group Ltd., Männedorf, Switzerland).

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

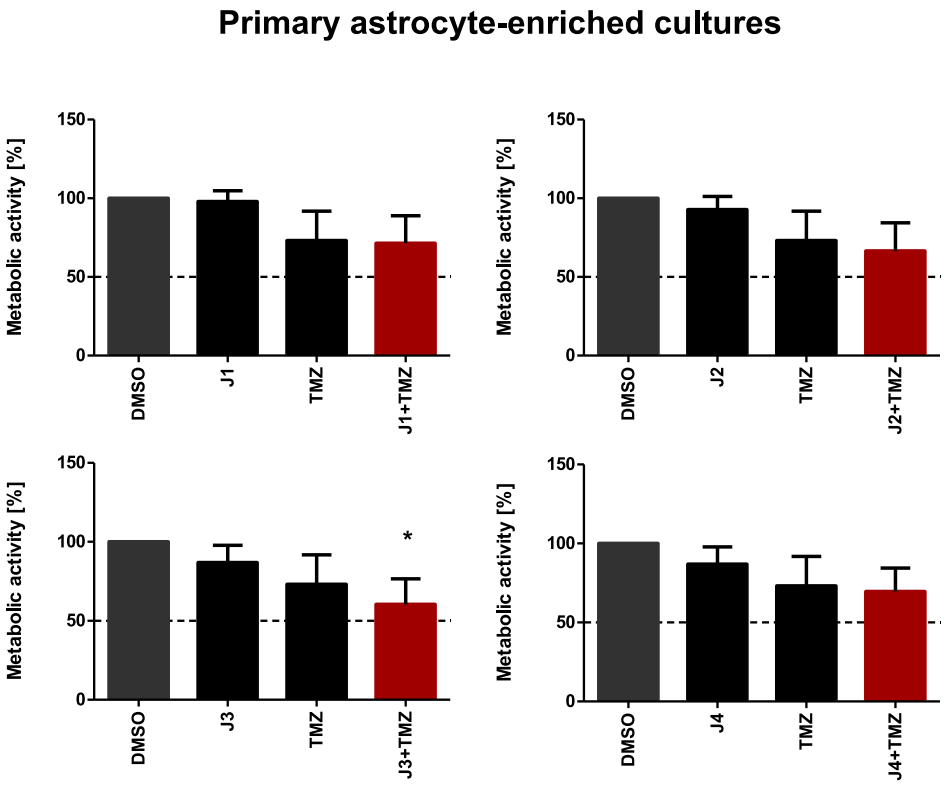


Figure 6. Metabolic activity in primary astrocyte-enriched cultures. For three days, primary astrocyte-enriched cultures were treated with J1, J2, J4, or TMZ or J1, J2, or J4 in combination with TMZ. Then, the medium was removed, cells were treated with the respective components for three additional days, and cell viability was measured. The DMSO sample served as a vehicle control. The MTT assay was performed in triplicate; the summary of three independent experiments (n = 3) is shown.

Table 1

U87		
J1 + TMZ	1.034	additive
J1 + Dinaciclib	1.076	additive
J2 + TMZ	0.894	antagonistic
J2 + Dinaciclib	0.987	additive
J3 + TMZ	0.930	additive
J3 + Dinaciclib	1.049	additive
J4 + TMZ	0.917	additive
J4 + Dinaciclib	1.060	additive

SC40		
J1 + TMZ	0.968	additive
J1 + Dinaciclib	0.945	additive
J2 + TMZ	1.039	additive
J2 + Dinaciclib	1.036	additive
J3 + TMZ	1.116	synergistic
J3 + Dinaciclib	1.126	synergistic
J4 + TMZ	1.141	synergistic
J4 + Dinaciclib	1.051	additive

PC40		
J1 + TMZ	1.012	additive
J1 + Dinaciclib	1.051	additive
J2 + TMZ	0.963	additive
J2 + Dinaciclib	1.046	additive
J3 + TMZ	0.965	additive
J3 + Dinaciclib	1.127	synergistic
J4 + TMZ	1.235	synergistic
J4 + Dinaciclib	1.164	synergistic

antagonistic	x < 0.9
additive	x > 0.9 and x < 1.1
synergistic	x > 1.1

4.4. Optical Microscopy. Images were taken using a PrimoVert microscope combined with an AxioCam ICc1 camera (Zeiss AG, Oberkochen, Germany) and documented.

4.5. Statistical Analysis. Data are presented as the mean ± standard error of the mean, and the statistical analysis was assessed by one-way ANOVA with Bonferroni correction, which was considered to be significant when $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), or $P < 0.0001$ (****) by using Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA). In addition, EC_{50} values were calculated (Prism 6, GraphPad Software, Inc., La Jolla, CA, USA).

The expected response to the combination treatment was calculated as fractional response to drug A (F_a) + fractional response to drug B (F_b) − ($F_a \times F_b$). Bliss (Excel) analysis was conducted to detect synergistic (ratio of the actual total response and the expected total response > 1.1), additive (this quotient equaled 0.9 to 1.1), or antagonistic effects (quotient < 0.9).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06169.

EC_{50} values were calculated based on the experimental procedure. Metabolic activity in human glioblastoma cell line T98G was determined by MTT assay. Cells were cultured with J1, J2, J4, or TMZ or J1, J2, or J4 in combination with TMZ. Concentrations of diisothiocyanate-derived mercapturic acids were selected based on the data obtained from U87 cell (S1) and used for T98G (left panel) or in excess (right panel). The DMSO

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

sample served as a vehicle control, and MTT assay was performed in triplicate; three independent experiments (n = 3) (PDF)

AUTHOR INFORMATION

Corresponding Author

Timo Burster − *Department of Biology, School of Sciences and Humanities, Nazarbayev University, 010000 Nur-Sultan, Kazakhstan Republic;* orcid.org/0000-0002-9596-6558; Email: timo.burster@nu.edu.kz

Authors

Pengfei Xu − *Department of General and Visceral Surgery, Surgery Center, Ulm University Medical Center, 89081 Ulm, Germany*

Mike-Andrew Westhoff − *Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, 89081 Ulm, Germany*

Amina Hadzalic − *Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, 89081 Ulm, Germany*

Klaus-Michael Debatin − *Department of Pediatrics and Adolescent Medicine, Ulm University Medical Center, 89081 Ulm, Germany*

Lukasz Winiarski − *Faculty of Chemistry, Division of Medicinal Chemistry and Microbiology, Wroclaw University of Science and Technology, 50-370 Wrocław, Poland*

Jozef Oleksyszyn − *Faculty of Chemistry, Division of Medicinal Chemistry and Microbiology, Wroclaw University of Science and Technology, 50-370 Wrocław, Poland*

Christian Rainer Wirtz − *Department of Neurosurgery, Ulm University Medical Center, 89081 Ulm, Germany*

Uwe Knippschild − *Department of General and Visceral Surgery, Surgery Center, Ulm University Medical Center, 89081 Ulm, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c06169

Author Contributions

A.H. carried out the experiments. P.X., U.K., L.W., J.O., C.R.W., and K.M.D. carried out data analysis work, interpreted the results, and/or synthesized components. TB designed experiments. T.B. and M.-A.W. interpreted data and wrote the manuscript. U.K. and P.X. edited the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

T.B. was funded by the Nazarbayev University Faculty-Development Competitive Research Grants Program, reference: 280720FD1907.

ABBREVIATIONS

ITCs, isothiocyanates; PCs, SC-derived differentiated/adherent glioblastoma cells; ROS, reactive oxygen species; SCs, sphere-cultured stem cell-enriched glioblastoma cell populations; TMZ, temozolomide

REFERENCES

(1) Jue, T. R.; McDonald, K. L. The challenges associated with molecular targeted therapies for glioblastoma. *J. Neuro Oncol.* **2016**, *127*, 427−434.

(2) Neftel, C.; Laffy, J.; Filbin, M. G.; Hara, T.; Shore, M. E.; Rahme, G. J.; Richman, A. R.; Silverbush, D.; Shaw, M. L.; Hebert, C. M.; Dewitt, J.; Gritsch, S.; Perez, E. M.; Gonzalez Castro, L. N.; Lan, X.; Druck, N.; Rodman, C.; Dionne, D.; Kaplan, A.; Bertalan, M. S.; Small, J.; Pelton, K.; Becker, S.; Bonal, D.; Nguyen, Q.-D.; Servis, R. L.; Fung, J. M.; Mylvaganam, R.; Mayr, L.; Gojo, J.; Haberler, C.; Geyeregger, R.; Czech, T.; Slavc, I.; Nahed, B. V.; Curry, W. T.; Carter, B. S.; Wakimoto, H.; Brastianos, P. K.; Batchelor, T. T.; Stemmer-Rachamimov, A.; Martinez-Lage, M.; Frosch, M. P.; Stamenkovic, I.; Riggi, N.; Rheinbay, E.; Monje, M.; Rozenblatt-Rosen, O.; Cahill, D. P.; Patel, A. P.; Hunter, T.; Verma, I. M.; Ligon, K. L.; Louis, D. N.; Regev, A.; Bernstein, B. E.; Tirosh, I.; Suvà, M. L. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. *Cell* **2019**, *178*, 835−849.

(3) Bunse, L.; Pusch, S.; Bunse, T.; Sahm, F.; Sanghvi, K.; Friedrich, M.; Alansary, D.; Sonner, J. K.; Green, E.; Deumelandt, K.; Kilian, M.; Neftel, C.; Uhlig, S.; Kessler, T.; von Landenberg, A.; Berghoff, A. S.; Marsh, K.; Steadman, M.; Zhu, D.; Nicolay, B.; Wiestler, B.; Breckwoldt, M. O.; Al-Ali, R.; Karcher-Bausch, S.; Bozza, M.; Oezen, I.; Kramer, M.; Meyer, J.; Habel, A.; Eisel, J.; Poschet, G.; Weller, M.; Preusser, M.; Nadji-Ohl, M.; Thon, N.; Burger, M. C.; Harter, P. N.; Ratliff, M.; Harbottle, R.; Benner, A.; Schrimpf, D.; Okun, J.; Herold-Mende, C.; Turcan, S.; Kaulfuss, S.; Hess-Stumpp, H.; Bieback, K.; Cahill, D. P.; Plate, K. H.; Hänggi, D.; Dorsch, M.; Suvá, M. L.; Niemeyer, B. A.; von Deimling, A.; Wick, W.; Platten, M. Suppression of antitumor T cell immunity by the oncometabolite (R)-2-hydroxyglutarate. *Nat. Med.* **2018**, *24*, 1192−1203.

(4) Chen, Z.; Wang, Z.; Pang, J. C.; Yu, Y.; Bieerkehazhi, S.; Lu, J.; Hu, T.; Zhao, Y.; Xu, X.; Zhang, H.; Yi, J. S.; Liu, S.; Yang, J. Multiple CDK inhibitor dinaciclib suppresses neuroblastoma growth via inhibiting CDK2 and CDK9 activity. *Sci. Rep.* **2016**, *6*, 29090.

(5) Dinkova-Kostova, A. T.; Kostov, R. V. Glucosinolates and isothiocyanates in health and disease. *Trends Mol. Med.* **2012**, *18*, 337−347.

(6) Singh, S. V.; Singh, K. Cancer chemoprevention with dietary isothiocyanates mature for clinical translational research. *Carcinogenesis* **2012**, *33*, 1833−1842.

(7) Trachootham, D.; Zhou, Y.; Zhang, H.; Demizu, Y.; Chen, Z.; Pelicano, H.; Chiao, P. J.; Achanta, G.; Arlinghaus, R. B.; Liu, J.; Huang, P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by β-phenylethyl isothiocyanate. *Cancer Cell* **2006**, *10*, 241−252.

(8) Grzywa, R.; Winiarski, Ł.; Psurski, M.; Rudnicka, A.; Wietrzyk, J.; Gajda, T.; Oleksyszyn, J. Synthesis and biological activity of diisothiocyanate-derived mercapturic acids. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 667−671.

(9) Cwiklowska, K.; Westhoff, M. A.; Freisinger, S.; Dwucet, A.; Halatsch, M. E.; Knippschild, U.; Debatin, K. M.; Schirmbeck, R.; Winiarski, L.; Oleksyszyn, J.; Wirtz, C. R.; Burster, T. Viability of glioblastoma stem cells is effectively reduced by diisothiocyanate-derived mercapturic acids. *Oncol. Lett.* **2018**, *16*, 6181−6187.

(10) Pruss, M.; Dwucet, A.; Tanriover, M.; Hlavac, M.; Kast, R. E.; Debatin, K.-M.; Wirtz, C. R.; Halatsch, M.-E.; Siegelin, M. D.; Westhoff, M.-A.; Karpel-Massler, G. Dual metabolic reprogramming by ONC201/TIC10 and 2-Deoxyglucose induces energy depletion and synergistic anti-cancer activity in glioblastoma. *Br. J. Cancer* **2020**, *122*, 1146−1157.

(11) Portnow, J.; Badie, B.; Chen, M.; Liu, A.; Blanchard, S.; Synold, T. W. The neuropharmacokinetics of temozolomide in patients with resectable brain tumors: potential implications for the current approach to chemoradiation. *Clin. Cancer Res.* **2009**, *15*, 7092−7098.

(12) Schneider, M.; Ströbele, S.; Nonnenmacher, L.; Siegelin, M. D.; Tepper, M.; Stroh, S.; Hasslacher, S.; Enzenmüller, S.; Strauss, G.; Baumann, B.; Karpel-Massler, G.; Westhoff, M.-A.; Debatin, K.-M.; Halatsch, M.-E. A paired comparison between glioblastoma "stem cells" and differentiated cells. *Int. J. Cancer* **2016**, *138*, 1709−1718.

(13) Strobel, H.; Baisch, T.; Fitzel, R.; Schilberg, K.; Siegelin, M. D.; Karpel-Massler, G.; Debatin, K. M.; Westhoff, M. A. Temozolomide

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936

and Other Alkylating Agents in Glioblastoma Therapy. *Biomedicines* **2019**, *7*, 69.
(14) Burster, T.; Traut, R.; Yermekkyzy, Z.; Mayer, K.; Westhoff, M.-A.; Bischof, J.; Knippschild, U. Critical View of Novel Treatment Strategies for Glioblastoma: Failure and Success of Resistance Mechanisms by Glioblastoma Cells. *Front. Cell Dev. Biol.* **2021**, *9*, 695325.
(15) Burster, T.; Gärtner, F.; Bulach, C.; Zhanapiya, A.; Gihring, A.; Knippschild, U. Regulation of MHC I Molecules in Glioblastoma Cells and the Sensitizing of NK Cells. *Pharmaceuticals* **2021**, *14*, 236.
(16) Herbener, V. J.; Burster, T.; Goreth, A.; Pruss, M.; von Bandemer, H.; Baisch, T.; Fitzel, R.; Siegelin, M. D.; Karpel-Massler, G.; Debatin, K. M.; Westhoff, M. A.; Strobel, H. Considering the Experimental use of Temozolomide in Glioblastoma Research. *Biomedicines* **2020**, *8*, 151.
(17) Saqub, H.; Proetsch-Gugerbauer, H.; Bezrookove, V.; Nosrati, M.; Vaquero, E. M.; de Semir, D.; Ice, R. J.; McAllister, S.; Soroceanu, L.; Kashani-Sabet, M.; Osorio, R.; Dar, A. A. Dinaciclib, a cyclin-dependent kinase inhibitor, suppresses cholangiocarcinoma growth by targeting CDK2/5/9. *Sci. Rep.* **2020**, *10*, 18489.
(18) Jane, E. P.; Premkumar, D. R.; Cavaleri, J. M.; Sutera, P. A.; Rajasekar, T.; Pollack, I. F. Dinaciclib, a Cyclin-Dependent Kinase Inhibitor Promotes Proteasomal Degradation of Mcl-1 and Enhances ABT-737-Mediated Cell Death in Malignant Human Glioma Cell Lines. *J. Pharmacol. Exp. Ther.* **2016**, *356*, 354−365.
(19) Ströbele, S.; Schneider, M.; Schneele, L.; Siegelin, M. D.; Nonnenmacher, L.; Zhou, S.; Karpel-Massle, G.; Westhoff, M.-A.; Halatsch, M.-E.; Debatin, K.-M. A Potential Role for the Inhibition of PI3K Signaling in Glioblastoma Therapy. *PLoS One* **2015**, *10*, No. e0131670.
(20) Westhoff, M.-A.; Zhou, S.; Nonnenmacher, L.; Karpel-Massler, G.; Jennewein, C.; Schneider, M.; Halatsch, M.-E.; Carragher, N. O.; Baumann, B.; Krause, A.; Simmet, T.; Bachem, M. G.; Wirtz, C. R.; Debatin, K.-M. Inhibition of NF-kappaB signaling ablates the invasive phenotype of glioblastoma. *Mol. Cancer Res.* **2013**, *11*, 1611−1623.

https://doi.org/10.1021/acsomega.1c06169
ACS Omega 2022, 7, 5929−5936