Original Article

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Identification of novel small-molecule inhibitors of glioblastoma cell growth and invasion by high-throughput screening

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Summary **Glioblastoma multiforme (GBM) is the most common and lethal type of primary brain tumor with a very poor prognosis. Current therapies for GBM remain palliative and advances made in decades have resulted in only a slight improvement in treatment outcome. Exploring new therapeutic agents for GBM treatment, therefore, is of prime importance. In the present study, we performed a high-throughput screening for GBM cell growth and invasion, with an attempt to identify novel potential anti-GBM agents. An annotated compound library (LOPAC1280) of 1,280 pharmacologically active compounds was screened and ten compounds were validated and identified as inhibitors of GBM cell growth and invasion. Four of them, *i.e.*, 6-nitroso-1,2-benzopyrone, *S*-(*p*-azidophenacyl) glutathione, phenoxybenzamine hydrochloride, and SCH-28080 have not been implicated in GBM cell growth and invasion previously, suggesting that they may serve as novel potential therapeutic agents for GBM treatment. In conclusion, novel inhibitors of GBM cell growth and invasion were identified in the present study, which provides a basis for the development of therapies for GBM, and may shed light on the molecular mechanisms underlying GBM cell behavior.**

Keywords: Glioblastoma, screening, annotated compound library, cell growth, cell invasion

1. Introduction

Glioblastoma multiforme (GBM) is the most common and most aggressive malignant brain tumor. Current therapeutic approaches for GBM are predominantly palliative, including maximal surgical resection, radiotherapy, and chemotherapy (*1*). However, one of the most important barriers to successful therapy is the diffuse invasion of GBM cells, making them elusive targets for effective surgical management and giving rise to tumor recurrence. Besides, lack of a significant antitumor effect from traditional therapies is frequently observed (*2*), resulting in quite low relative survival (five-year survival rates are less than 5%) (The Central Brain Tumor Registry of the United States, 2012). Therefore, identifying novel effective therapeutic agents for treating GBM is urgently needed.

Etiology and pathophysiology of GBM are complex and currently unclear. Potential molecular targets for GBM therapies are being explored while effective ones are still largely unknown. As GBM is characterized by high proliferation rate and diffuse invasion, regulators targeting these cell behaviors may lead to immediate clinical improvement. The development of experimental technologies to measure cell growth or invasion has made it possible to acquire potential anticancer compounds, while a major obstacle

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is that the current cell invasion assays are difficult to perform in parallel at the scale required to screen large libraries (*3*). High-throughput approaches, which allow simultaneous screening of hundreds to thousands of compounds, are therefore of interest to basic scientists and those in search of therapeutics (*4*). Bioluminescent assays, which are based on the detection of ATP by the luciferin-luciferase reaction, are being studied for high-throughput screening due to their sensitivity, broad linearity, and robustness to library compounds and complex biological samples (*5*). A bioluminescent-based assay for GBM cell growth and invasion was developed in our laboratory previously (*6*), and it was adapted here to allow the possibility of screening regulators of GBM cell behavior in a high-throughput manner.

In the present study, using a high-throughput screening approach, we screened an annotated compound library (LOPAC1280) of 1,280 pharmacologically active compounds that influence most cellular processes and cover all major drug target classes, to identify novel potential therapeutic agents against GBM. LOPAC1280 represents a collection of compounds from 56 pharmacological classes with well-characterized activities and experimentally validated biological mechanisms, and thus, this screening may also provide new insights into the molecular basis of GBM.

2. Materials and Methods

2.1. *Cells, cell cultures and compounds*

Human glioblastoma cell lines U87MG and U251MG were obtained from American Type Culture Collection (Manassas, VA, USA) and maintained in DMEM supplemented with 10% fetal bovine serum (FBS). U87-luc (U87MG glioblastoma cells genetically engineered to express firefly luciferase reporter gene) was kindly provided by Santosh Kesari (Dana-Farber Cancer Institute, Harvard Medical University) and cultured in DMEM containing 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, and 0.5 mg/mL G418 (Sigma-Aldrich, St. Louis, MO, USA). Cells were maintained in a humidified tissue culture incubator at 37°C with a 5% CO_2 atmosphere.

Compounds from LOPAC1280 (Sigma-Aldrich, St. Louis, MO, USA) were received as 10 mM DMSO stock solutions and stored at –20°C until further use. For validation experiments, active compounds (*i.e.*, "hits") from the primary high-throughput screening were prepared at a concentration ranging between 0.01 and 100 μM.

2.2. *High-throughput screening of LOPAC1280*

For primary screening, a high-throughput screening assay for cell growth and invasion was performed and a library of 1,280 compounds was screened. Briefly, the luciferin-immobilized 96-well black-wall clear-bottom plates (Becton Dickinson, Franklin Lakes, NJ, USA) were prepared following the procedure described in our previous paper (*6*) (Figure 1A). For each plate, the top half (rows A-D) was not coated with luciferin, with the aim of evaluating cell proliferation. Wells in rows E-H were coated with luciferin on the bottom to evaluate cell invasion. Each compound of the LOPAC1280 library was mixed with U87-Luc cell conditioned media and Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA, USA), which had been widely used in tumor cell invasion assays or functioned as 3D cell culture platforms (*7,8*), to a final concentration of 10 μM (the DMSO concentration was 0.1% for all samples and controls). Then the mixture was added onto the 96-well plates in triplicate in a cold room at 4°C. Each 96-well plate was placed in an incubator at 37°C for 2 h. Next, luciferin (1 μL) was placed in each of the wells in rows A-D. About 5,000 U87-Luc cells in 5 μL media were loaded onto the Matrigel (0 h) in all wells followed by incubation in a humidified incubator at 37°C in a 5% CO_2 atmosphere for 24 h. The bioluminescent signals were detected at 0 h and 24 h using the IVIS 100 system (Xenogen Co., Alameda, CA, USA). Signal intensity was quantified using Living Image software (version 2.50.1, Xenogen Co., Alameda, CA, USA) (in terms of photon counts).

A good linear correlation between the U87-Luc cell number in Matrigel and bioluminescent signal had been obtained in our previous study (*6*). The photon counts of wells in rows A-D (no luciferin coating) reflected the total number of cells and those in rows E-H (luciferin coated) reflect the cells that moved through the Matrigel. Accordingly, cell growth and invasion were measured. For each 96-well plate, 15 compounds were evaluated. Z' factor was used for quality control of the screening plates, which was defined as described in the following equation: $1 - (3\sigma s + 3\sigma b)/|\mu s - \mu b|$, where σ represents the S.D. of signal (σs) or background (σb) and l is the mean and μ represents the mean (*9*).

2.3. *Cell growth assay*

Cell growth was assessed by CellTiter 96® AQueous cell proliferation assay (MTS) (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, 10,000 U87MG or U251MG cells in 100 μL medium were seeded in triplicate in each well of the 96-well white-wall clear-bottom plates and incubated overnight, followed by 24 h treatment with active compounds at different concentrations (0.01 μM to 100 μM). Cells were then washed and fresh medium was added followed by Cell Titer 96® AQueous One solution (20 μL/well). After 3 h incubation, absorbance at 490 nm was measured using the FLUOstar Omega Microplate Reader (BMG LABTECH, Cary, NC,

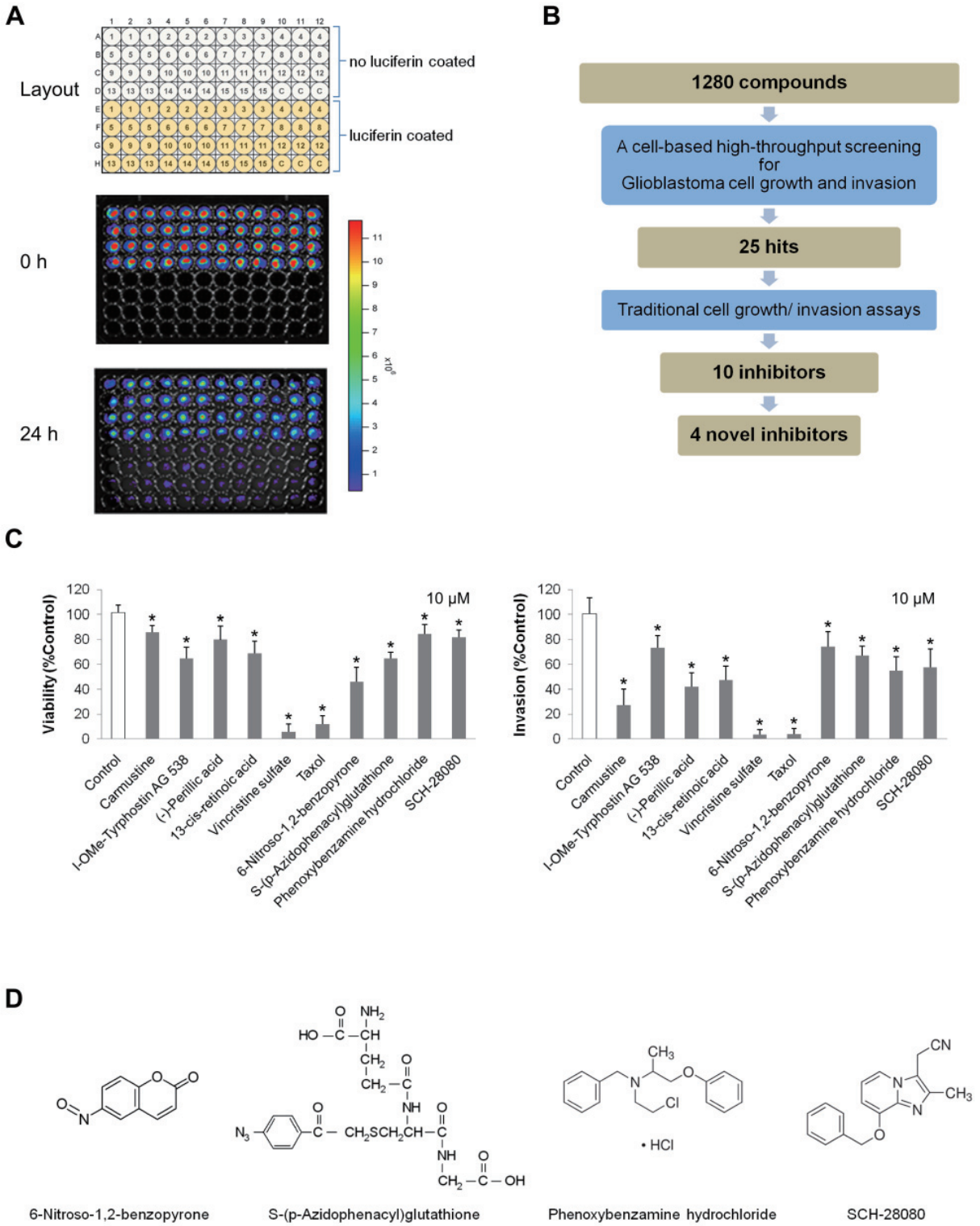

Figure 1. Results of LOPAC1280 high-throughput screening for anti-GBM compounds and chemical structures of the validated novel hits. (A) Layout of the 96-well plate for LOPAC1280 high-throughput screening. Wells in rows A-D have no luciferin coating. U87-Luc cells and luciferin were added simultaneously and cell growth was monitored. Bottom of wells in rows E-H were coated with luciferin. U87-Luc cells alone were added and the bioluminescent signals could be detected only if the cells invade the Matrigel and reached the bottom of wells. Columns 10-12, rows D and H, control cells treated with 0.1% DMSO; Other wells, cells treated with LOPAC1280 compounds (10 μM). Relative luciferase activity of the control was defined as 100% and measurements from other wells were calculated accordingly. For each 96-well plate, 15 compounds were evaluated for their activity in GBM cell growth and invasion. **(B)** Results: 1,280 compounds were screened in triplicate, leading to an initial list of 25 active compounds, of which ten were subsequently validated in traditional assays. The ten validated compounds and their effects on U87-Luc cell growth and invasion as evaluated by the high-throughput screening are shown in **(C)**. Values in bar graphs represent mean ± S.D. of three independent experiments. * $p < 0.05$ *vs.* control. Four (*i.e.*, 6-nitroso-1,2-benzopyrone, *S*-(*p*-azidophenacyl) glutathione, phenoxybenzamine hydrochloride, and SCH-28080) of the ten validated compounds were identified as novel inhibitors of GBM cell growth and invasion. Their chemical structures are shown in **(D)**.

USA).

2.4. *Cell invasion assay*

Cell invasive capacity was evaluated by 24-well Matrigel™ Invasion Chamber according to the manufacturer's instructions (BD Biosciences). Briefly, 25,000 U87MG or U251MG cells in 500 μL medium containing active compounds at different concentrations were added in triplicate to the inserts and incubated for 24 h. After the removal of non-invading cells with a cotton swab, cells on the lower surface of the membrane were stained with a Diff-Quik stain kit (Dade-Behring, Newark, DE, USA). Cells were counted in five chosen microscope fields in each well and the results were numerically averaged. Cell invasion was calculated according to the manufacturer's manual.

2.5. *Cell migration assay*

Cell migration was examined by wound-healing assay as described previously (*10*). Relative migration distance was quantified by measuring the advance of the two opposing wound edges at 0 h and 12 h using Slidebook software (Intelligent Imaging Innovations, Inc., Denver, CO, USA).

2.6. *Statistical analysis*

Data are presented as the mean ± S.D. Statistical significance was determined by one-way ANOVA, followed by Dunnett's *post-hoc* test. All tests were 2-sided and a value of $p < 0.05$ was considered significant. Statistical analyses were performed using GraphPad Prism for Windows (Graphpad Software, San Diego, CA, USA).

3. Results

3.1. *High-throughput screening for novel inhibitors of GBM cell growth and invasion of LOPAC1280*

As an initial step to discover novel potential compounds against GBM, 1,280 pharmacologically active compounds from 56 pharmacological classes were quantitatively assessed for their effects on GBM cell growth and invasion at a concentration of 10 μM (Figure 1A). Assay quality was evaluated using Z′-factors (see Materials and Methods). A Z′-factor of 1 is considered ideal; a Z′-factor between 0.5 and 1.0 represented excellent assays (*9*). For the screen plates, an average Z′ factor of 0.513 was obtained, indicating that the assay was acceptable for cell-based screening (*11*). Outlier wells caused by plate preparation misdispensing were not included in the analysis.

The high-throughput screening of LOPAC1280 resulted in an initial identification of 25 inhibitors (Figure 1B). As will be shown below, ten of them were validated in traditional cell growth, invasion and migration assays. They belong to several pharmacological classes, suggesting that these classes should be paid more attention to in the development of therapies for GBM. It is gratifying that carmustine, I-OMe-Tyrphostin AG 538, (–)-perillic acid, 13-*cis*-retinoic acid, vincristine sulfate, and Taxol, which had been studied in preclinical experiments, evaluated in clinical trials, or used in clinical as chemotherapeutic agents for tumors including GBM (see Discussion for details), were identified in our screening (Table 1). The other four, *i.e.*, 6-nitroso-1,2-benzopyrone, *S*-(*p*-azidophenacyl) glutathione, phenoxybenzamine hydrochloride and SCH-28080, had not been implicated in GBM cell behaviors and were therefore identified as novel potential anti-GBM agents (Table 1, Figure 1D).

Table 1. Potential hit compounds from LOPAC1280 high-throughput screening

LOPAC Cat. #	Name of compound	Pharmacological class	Activity
C 0400	Carmustine	DNA	DNA alkylating agent; causes interstrand crosslinks
T 7697	I-OMe-Tyrphostin AG 538	Phosphorylation	Insulin growth factor 1 (IGF-1) receptor protein tyrosine kinase inhibitor
P 7083	(–)-Perillic acid	G protein	Interferes with activity of p21 ras and other small G proteins by inhibiting post-translational cysteine isoprenylation
R 3255	13-*cis*-Retinoic acid	Transcription	Anti-inflammatory and antitumor actions mediated through RAR-beta and RAR-alpha receptors
V 8879	Vincristine sulfate	Cytoskeleton and ECM	Inhibitor of microtubule assembly
T 7402	Taxol	Cytoskeleton and ECM	Antitumor agent; promotes assembly of microtubules and inhibits tubulin disassembly process
N 8403	6-Nitroso-1,2-benzopyrone	Transcription	Poly (ADP-ribose) polymerase (PARP) ligand which preferentially destabilizes one of the two zinc-fingers, inactivating the enzyme
A 1782	*S*-(*p*-Azidophenacyl) glutathione	Multi-drug resistance	Glyoxalase and glutathione S-transferase inhibitor
B-019	Phenoxybenzamine hydrochloride	Adrenoceptor	Selective alpha adrenoceptor blocking agent; calmodulin antagonist
S 4443	SCH-28080	Ion channels	Potent inhibitor of gastric H^+ and K^+-ATPase.

3.2. *Effects of the active compounds on GBM cell growth*

A traditional cell viability assay (MTS assay) was performed to achieve a dose-response evaluation of the active compounds in two GBM cell lines, U87MG and U251MG. The concentration used in the primary screening was 10 μM (see Materials and Methods). In this part, a concentration ranging between 0.01 and 100 μM was applied (Figure 2). All the four compounds caused a dose-dependent inhibition of GBM cell growth, although the degree of inhibition varied.

3.3. *Effects of the active compounds on GBM cell invasion*

The invasive potential of GBM cells was tested using a Matrigel cell invasion assay and effects of the four novel inhibitors are shown (Figure 3). Compared to the control, 1 μM 6-nitroso-1,2-benzopyrone, 1 μM *S*-(*p*-azidophenacyl) glutathione, 10 μM phenoxybenzamine hydrochloride and 10 μM SCH-28080 significantly reduced the invasion of U87MG cells to 79.8%, 65.2%, 48.6%, and 52.9%, respectively. A similar result was obtained in U251MG cells.

3.4. *Effects of the active compounds on GBM cell migration*

Additionally, we examined whether the reduced invasiveness of GBM cells caused by the hit compounds was accompanied by reduced cell motility. A cell migration assay (wound healing assay) was employed (Figure 4). As shown, compared to the control, 1 μM 6-nitroso-1,2-benzopyrone and 1 μM *S*-(*p*-azidophenacyl) glutathione significantly reduced U87MG cell migration to 86% and 80%, respectively. When treated with 10 μM phenoxybenzamine hydrochloride or 10 μM SCH-28080, U87MG cell migration was reduced to 72% or 82%, respectively. The decreases in U251MG cell migration were also

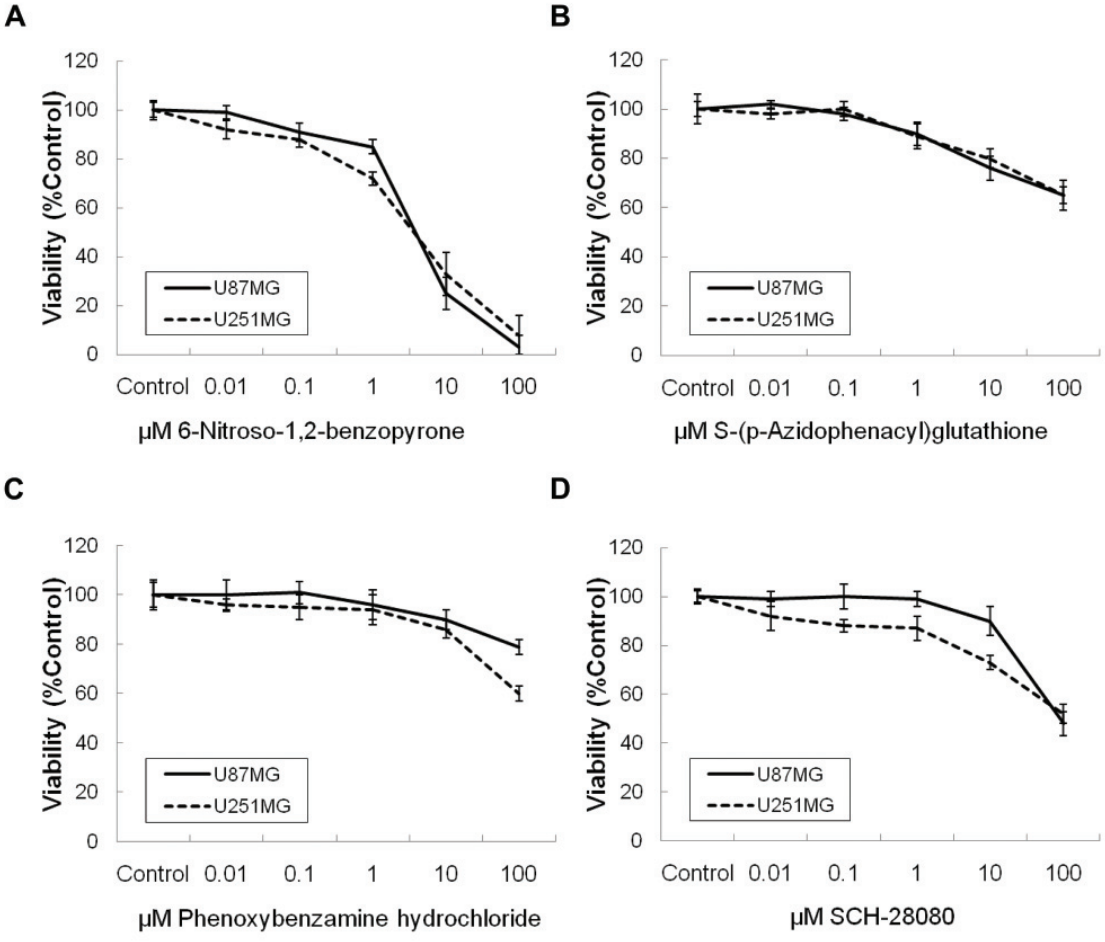

Figure 2. Dose-response curves displaying the activity of the four active compounds in GBM cells. Two GBM cell lines (U87MG and U251MG) were treated with various concentrations of 6-nitroso-1,2-benzopyrone **(A)** *S*-(*p*-azidophenacyl) glutathione **(B)** phenoxybenzamine hydrochloride **(C)** and SCH-28080 **(D)** for 24 h. Cell growth was measured by MTS cell viability assay. Cell viability was calculated as the ratio of the corrected absorbance value of the treated cells to that of untreated control cells. The results are presented as mean ± S.D. of three independent experiments.

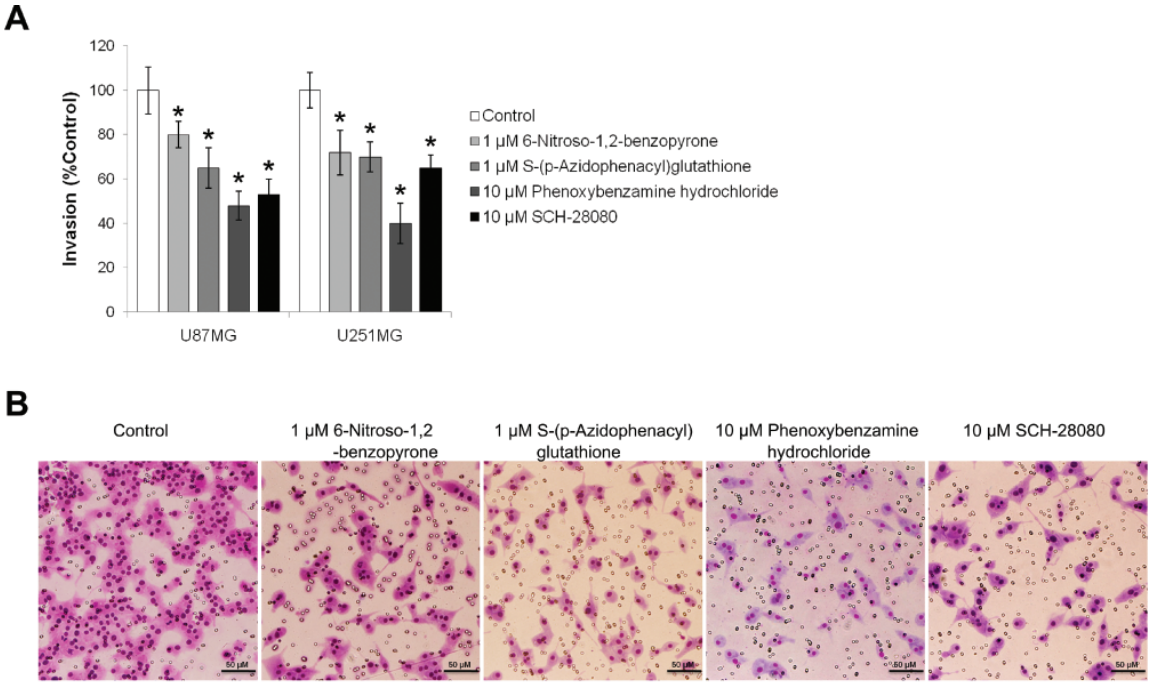

Figure 3. Effects of the four active compounds on GBM cell invasion. (A) U87MG and U251MG cells were treated with the active compounds from the primary high-throughput screening for 24 h. Cell invasion was evaluated by Matrigel cell invasion assay. The invaded cells that adhered to the lower surface of the membrane were counted. The invasive potential of the treated cells is presented as a percentage invasion of control. Data represent mean ± S.D. of three independent experiments. * $p < 0.05$ *vs.* control. **(B)** The representative images of U87MG cells are shown. Scale bar, 50 μm.

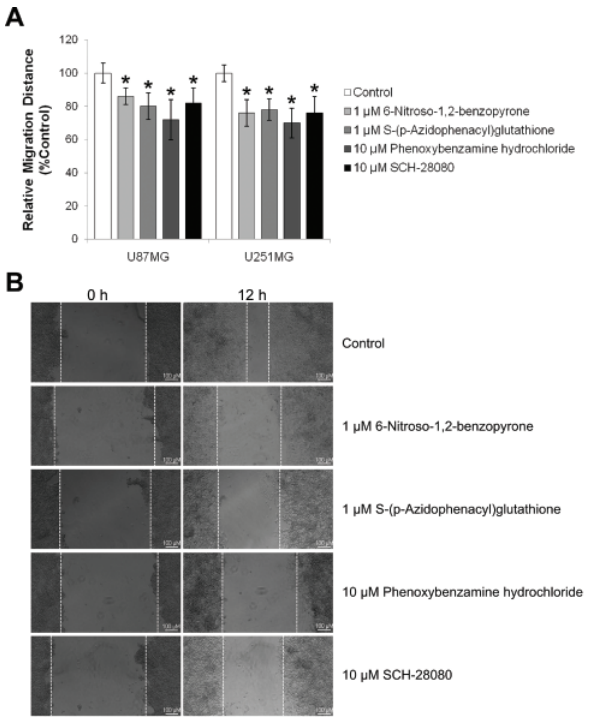

Figure 4. Effects of the four active compounds on GBM cell migration. (A) U87MG and U251MG cells were treated with the active compounds from the primary high-throughput screening for 12 h, and cell migration was measured by wound-healing assay. Cell migration is presented as percentage of relative migration distance divided by that of the control. Each value represents the mean ± S.D. of three independent experiments. * $p < 0.05$ *vs.* control. **(B)** The representative images of U87MG cells are shown. Scale bar, 100 μm.

significant. In other words, as expected, all four compounds caused a statistically significant reduction in the cell migration.

Parallel experiments for evaluating effects of different concentrations of DMSO (used in this study) on GBM cell proliferation, invasion, and migration were performed to rule out the possibility that DMSO might have subtle effects on GBM cell behaviors. No significant difference was observed after DMSO treatment in each experiment (data not shown).

4. Discussion

Mechanisms underlying GBM cell proliferation, invasion, and migration are very complicated. Regulators of these biological behaviors, which hold the promise of effective therapeutic approaches for treating GBM, still have not been well defined. In this study, all compounds of the LOPAC1280, which is a versatile library including the latest drug-like molecules in the fields of cell signaling and neuroscience, and reflects the most commonly screened targets in the drug discovery community, were evaluated in the primary screening assay. The four newly identified potential anti-GBM compounds come from distinct pharmacological classes, some of which have never been implicated in tumor cell behaviors suggesting that these classes may be involved in diverse biological processes of GBM. Other unrevealed compounds from

these classes may also be worth noting in terms of their anticancer effects.

In this study, ten validated active compounds were obtained, of which six have been previously reported to be implicated in GBM cell behaviors (Table 1). Carmustine is a DNA alkylating agent and causes interstrand crosslinks, and it has long been used as a treatment for GBM (*12*). I-OMe-Tyrphostin AG 538 is an insulin growth factor 1 (IGF-1) receptor (IGF-1R) protein tyrosine kinase inhibitor. IGF-1 has been well discussed in GBM (*13*), and targeting IGF-1R has been studied as a treatment option for GBM (*14*). (–)-Perillic acid is a metabolite of perillyl alcohol (POH), which has shown a therapeutic anticancer effect and is currently under phase I and II clinical trials for human cancers including GBM (*15,16*). 13-*cis*-Retinoic acid (cRA) has antitumor actions mediated through RAR-beta and RAR-alpha receptors, which has long been evaluated and has shown therapeutic effectiveness in clinical trials in treatment of brain tumors (*17*). Vincristine sulfate and Taxol, both from the class of cytoskeleton and ECM, are being clinically evaluated alone or combined for use against gliomas (*18,19*). The identification of these compounds is gratifying and may help validate this screen as an effective means to identify compounds that regulate GBM cell behavior. Moreover, the therapeutic effects of these compounds in clinical studies suggest that the four newly identified compounds in our study (*i.e.* 6-nitroso-1,2-benzopyrone, *S*-(*p*-azidophenacyl) glutathione, phenoxybenzamine hydrochloride, and SCH-28080), might benefit patients with GBM and are therefore worth further study (Table 1).

6-Nitroso-1,2-benzopyrone is a poly(ADP-ribose) polymerase (PARP) ligand that has been reported to suppress proliferation of leukemic and other malignant human cells (*20*), while few studies have focused on its effect on GBM cell behavior.

S-(*p*-Azidophenacyl) glutathione is a glyoxalase and glutathione S-transferase (GST) inhibitor. It has been reported that blockage of glyoxalase resulted in an inhibition of proliferation of human leukemia cells (*21*). GSTs have been implicated in the development of drug resistance (*22*), and may be responsible for poor response to alkylating agents in GBM treatment (*23*). In this study, the effects of *S*-(*p*-azidophenacyl) glutathione on GBM cell behavior were examined for the first time.

Phenoxybenzamine hydrochloride is a selective alpha adrenoceptor blocking agent as well as a calmodulin antagonist. The alpha adrenoceptor antagonist has been extensively studied in human prostate cancer and shown to be involved in cell cycle arrest and apoptosis (*24*), whereas it has rarely been studied in GBM cells. Based on the distribution of alpha-adrenoceptors in the human central nervous system, our findings are of concern (*25*). Besides, phenoxybenzamine hydrochloride also reacts irreversibly with calmodulin (*26*), which has been shown to play roles in astrocytoma cell growth (*27*). In this connection, our finding of the effects of phenoxybenzamine hydrochloride in GBM cell behavior is attractive.

SCH-28080 is a potent inhibitor of gastric H^+- and K^+-ATPase, which was considered to exert its antisecretory effect by a competitive interaction with the high affinity K^+-site of the gastric ATPase (*28*). It has been shown to inhibit basal and stimulated aminopyrine accumulation in isolated gastric glands, and to cause relaxation of human airway smooth muscle (*29,30*). Here SCH-28080 exhibited antitumor activity against GBM *in vitro*, whereas the underlying molecular mechanisms still need to be elucidated.

The four active compounds, which have not been previously implicated in GBM cell behavior, are all shown in our study to exert inhibitory effects on GBM cell growth and invasion. This favorable result allows them to be further studied as novel potential therapeutic agents against GBM.

It should be mentioned that although these compounds showed favorable effects on GBM cells *in vitro*, there is still a long way to go before they benefit patients. Their pharmacokinetic and pharmacodynamic activities need to be addressed, and their effectiveness crossing the blood-brain barrier and delivery to a brain tumor area are of vital concern (*31*). Good news is that scientists have never stopped devising innovative approaches to circumvent these obstacles, and strategies for increasing drug delivery are being discovered (*32*). These agents also need further investigation for their cancer-specific action because the toxicity of anticancer agents against normal cells is still one of the major challenges in cancer treatment (*33*). While these uncertainties could not stop this study from providing starting points for anti-GBM drug discovery and design.

In summary, *via* a high-throughput screening, from an annotated compound library, we identified four novel small-molecule inhibitors of GBM cell growth and invasion, with an attempt to provide useful therapeutic leads for GBM treatment. Currently, these four active compounds are being investigated in our laboratory to explore the underlying mechanisms, and their anti-tumor effects are also being evaluated in an intracranial GBM xenograft model. The results will be reported in detail elsewhere.

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References

1. Azizi SA, Miyamoto C. Principles of treatment of malignant gliomas in adults: An overview. J Neurovirol. 1998; 4:204-216.
2. Mariani L, Beaudry C, McDonough WS, Hoelzinger DB, Demuth T, Ross KR, Berens T, Coons SW, Watts G, Trent JM, Wei JS, Giese A, Berens ME. Glioma cell motility is associated with reduced transcription of proapoptotic and proliferation genes: A cDNA microarray analysis. J Neurooncol. 2001; 53:161-176.
3. Decaestecker C, Debeir O, Van Ham P, Kiss R. Can anti-migratory drugs be screened *in vitro*? A review of 2D and 3D assays for the quantitative analysis of cell migration. Med Res Rev. 2007; 27:149-176.
4. Henry S, Bigler S, Wang J. High throughput analysis of neural progenitor cell proliferation in adult rodent hippocampus. Biosci Trends. 2009; 3:233-238.
5. Fan F, Wood KV. Bioluminescent assays for high-throughput screening. Assay Drug Dev. Technol. 2007; 5:127-136.
6. Zhao H, Tang C, Cui K, Ang BT, Wong ST. A screening platform for glioma growth and invasion using bioluminescence imaging. Laboratory investigation. J Neurosurg. 2009; 111:238-246.
7. Justice BA, Badr NA, Felder RA. 3D cell culture opens new dimensions in cell-based assays. Drug Discov Today. 2009; 14:102-107.
8. Terranova VP, Hujanen ES, Loeb DM, Martin GR, Thornburg L, Glushko V. Use of a reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells. Proc Natl Acad Sci U S A. 1986; 83:465-469.
9. Zhang JH, Chung TD, Oldenburg KR. A Simple statistical parameter for use in evaluation and validation of high throughput screening assays. J Biomol Screen. 1999; 4:67-73.
10. Rajasekaran SA, Palmer LG, Quan K, Harper JF, Ball WJ Jr, Bander NH, Peralta Soler A, Rajasekaran AK. Na,K-ATPase beta-subunit is required for epithelial polarization, suppression of invasion, and cell motility. Mol Biol Cell. 2001; 12:279-295.
11. Quintavalle M, Elia L, Price JH, Heynen-Genel S, Courtneidge SA. A cell-based high-content screening assay reveals activators and inhibitors of cancer cell invasion. Sci Signal. 2011; 4:49.
12. Brandes AA, Tosoni A, Basso U, Reni M, Valduga F, Monfardini S, Amistà P, Nicolardi L, Sotti G, Ermani M. Second-line chemotherapy with irinotecan plus carmustine in glioblastoma recurrent or progressive after first-line temozolomide chemotherapy: A phase II study of the Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). J Clin Oncol. 2004; 22:4779-4786.
13. Schlenska-Lange A, Knüpfer H, Lange TJ, Kiess W, Knüpfer M. Cell proliferation and migration in glioblastoma multiforme cell lines are influenced by insulin-like growth factor 1 *in vitro*. Anticancer Res. 2008; 28:1055-1060.
14. Yin S, Girnita A, Stromberg T, Khan Z, Andersson S, Zheng H, Ericsson C, Axelson M, Nistér M, Larsson O, Ekström TJ, Girnita L. Targeting the insulin-like growth factor-1 receptor by picropodophyllin as a treatment option for glioblastoma. Neuro oncol. 2010; 12:19-27.
15. Azzoli CG, Miller VA, Ng KK, Krug LM, Spriggs DR, Tong WP, Riedel ER, Kris MG. A phase I trial of perillyl alcohol in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2003; 51:493-498.
16. Liu G, Oettel K, Bailey H, Ummersen LV, Tutsch K, Staab MJ, Horvath D, Alberti D, Arzoomanian R, Rezazadeh H, McGovern J, Robinson E, DeMets D, Wilding G. Phase II trial of perillyl alcohol (NSC 641066) administered daily in patients with metastatic androgen independent prostate cancer. Invest New Drugs. 2003; 21:367-372.
17. Jaeckle KA, Hess KR, Yung WK, Greenberg H, Fine H, Schiff D, Pollack IF, Kuhn J, Fink K, Mehta M, Cloughesy T, Nicholas MK, Chang S, Prados M; North American Brain Tumor Consortium. Phase II evaluation of temozolomide and 13-*cis*-retinoic acid for the treatment of recurrent and progressive malignant glioma: A North American Brain Tumor Consortium study. J Clin Oncol. 2003; 21:2305-2311.
18. Levin VA, Uhm JH, Jaeckle KA, Choucair A, Flynn PJ, Yung WKA, Prados MD, Bruner JM, Chang SM, Kyritsis AP, Gleason MJ, Hess KR. Phase III randomized study of postradiotherapy chemotherapy with alpha-difluoromethylornithine-procarbazine, *N*-(2-chloroethyl)-*N′*-cyclohexyl-*N*-nitrosurea, vincristine (DFMO-PCV) versus PCV for glioblastoma multiforme. Clin Cancer Res. 2000; 6:3878-3884.
19. Terzis AJ, Thorsen F, Heese O, Visted T, Bjerkvig R, Dahl O, Arnold H, Gundersen G. Proliferation, migration and invasion of human glioma cells exposed to paclitaxel (Taxol) *in vitro*. Br J Cancer. 1997; 75:1744-1752.
20. Rice WG, Hillyer CD, Harten B, Schaeffer CA, Dorminy M, Lackey DA 3rd, Kirsten E, Mendeleyev J, Buki KG, Hakam A, Rice WG. Induction of endonuclease-mediated apoptosis in tumor cells by C-nitroso-substituted ligands of poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A. 1992; 89:7703-7707.
21. Lo TW, Thornalley PJ. Inhibition of proliferation of human leukaemia 60 cells by diethyl esters of glyoxalase inhibitors *in vitro*. Biochem Pharmacol. 1992; 44:2357-2363.
22. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene. 2003; 22:7369-7375.
23. Juillerat-Jeanneret L, Bernasconi CC, Bricod C, Gros S, Trepey S, Benhattar J, Janzer RC. Heterogeneity of human glioblastoma: Glutathione-S-transferase and methylguanine-methyltransferase. Cancer Invest. 2008; 26:597-609.
24. Liou SF, Lin HH, Liang JC, Chen IJ, Yeh JL. Inhibition of human prostate cancer cells proliferation by a selective alpha1-adrenoceptor antagonist labedipinedilol-A involves cell cycle arrest and apoptosis. Toxicology. 2009; 256:13-24.
25. Zilles K, Qü M, Schleicher A. Regional distribution and heterogeneity of alpha-adrenoceptors in the rat and human central nervous system. J Hirnforsch. 1993; 34:123-132.
26. Ning YM, Sánchez ER. Evidence for a functional interaction between calmodulin and the glucocorticoid receptor. Biochem Biophys Res Commun. 1995; 208:48-54.
27. Lee GL, Hait WN. Inhibition of growth of C6 astrocytoma cells by inhibitors of calmodulin. Life Sci. 1985; 36:347-354.
28. Beil W, Hackbarth I, Sewing KF. Mechanism of gastric antisecretory effect of SCH 28080. Br J Pharmacol. 1986; 88:19-23.

29. Wallmark B, Briving C, Fryklund J, Munson K, Jackson R, Mendlein J, Rabon E, Sachs G. Inhibition of gastric H^+,K^+-ATPase and acid secretion by SCH 28080, a substituted pyridyl(1,2a)imidazole. J Biol Chem. 1987; 262:2077-2084.
30. Rhoden KJ, Tallini G, Douglas JS. H^+-K^+ ATPase inhibitors cause relaxation of guinea pig and human airway smooth muscle *in vitro*. J Pharmacol Exp Ther. 1996; 276:897-903.
31. Pardridge WM. The blood-brain barrier: Bottleneck in brain drug development. NeuroRx. 2005; 2:3-14.
32. Groothuis DR. The blood-brain and blood-tumor barriers: A review of strategies for increasing drug delivery. Neuro Oncol. 2000; 2:45-59.
33. Maeda H, Hori S, Ohizumi H, Segawa T, Kakehi Y, Ogawa O, Kakizuka A. Effective treatment of advanced solid tumors by the combination of arsenic trioxide and L-buthionine-sulfoximine. Cell Death Differ. 2004; 11:737-746.

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