**TABLES**

**Appendix Table S1** Selected papers for systems using artificial GBM TME

|  |  |
| --- | --- |
| **Titles** | **References** |
|  |
|  |
| A novel 3D nanofibre scaffold conserves the plasticity of glioblastoma stem cell invasion by regulating galectin-3 and integrin-β1 expression. | (52) |  |
| Glioma-astrocyte interactions on white matter tract-mimetic aligned electrospun nanofibers. |   (15) |  |
| Aligned Chitosan-Polycaprolactone Polyblend Nanofibers Promote the Migration of Glioblastoma Cells. |  (16) |  |
| Mimicking white matter tract topography using core–shell electrospun nanofibers to examine migration of malignant brain tumors | (17) |  |
| Electrospun nanofibrous scaffolds increase the efficacy of stem cell-mediated therapy of surgically resected glioblastoma. | (14) |  |
| Guiding intracortical brain tumour cells to an extracortical cytotoxic hydrogel using aligned polymeric nanofibers. |  (31) |  |
| Integration of microfluidic chip with biomimetic hydrogel for 3D controlling and monitoring of cell alignment and migration: Biomimetic Hydrogel. |  (32) |  |
| Multidimensional hydrogel models reveal endothelial network angiocrine signals increase glioblastoma cell number, invasion, and temozolomide resistance. |  (28) |  |
| Collagen Matrices Mediate Glioma Cell Migration Induced by an Electrical Signal. |  (29) |  |
| Bioengineered 3D Brain Tumor Model To Elucidate the Effects of Matrix Stiffness on Glioblastoma Cell Behavior Using PEG-Based Hydrogels. |  (26) |  |
| Glioblastoma Behaviors in Three-Dimensional Collagen-Hyaluronan Composite Hydrogels. |  (23) |  |
| Glioblastoma and cerebral organoids: development and analysis of an in vitro model for glioblastoma migration. |  (53) |  |

**Appendix Table S2** Selected papers for anti-migratory drugs

| **Titles** | **References** |
| --- | --- |
| The flavonoid rutin and its aglycone quercetin modulate the microglia inflammatory profile improving antiglioma activity. | (39) |
| Differential Effects of Wedelia chinensis on Human Glioblastoma Multiforme Cells. | (37) |
| Inhibition of TRPM7 by carvacrol suppresses glioblastoma cell proliferation, migration, and invasion. | (54) |
| Natural dietary compound naringin inhibits glioblastoma cancer neoangiogenesis. | (35) |
| Naringin suppresses cell metastasis and the expression of matrix metalloproteinases (MMP-2 and MMP-9) via the inhibition of ERK-P38-JNK signaling pathway in human glioblastoma. | (36) |
| Berberine inhibits glioma cell migration and invasion by suppressing TGF-beta1/COL11A1 pathway. | (55) |
| Kukoamine A inhibits human glioblastoma cell growth and migration through apoptosis induction and epithelial-mesenchymal transition attenuation. | (56) |
| Ginsenoside Rh2 inhibits metastasis of glioblastoma multiforme through Akt-regulated MMP13. | (41) |
| Verbascoside suppresses the migration and invasion of human glioblastoma cells via targeting c-Met-mediated epithelial-mesenchymal transition. | (57) |
| Curcumin piperidone derivatives induce anti-proliferative and anti-migratory effects in LN-18 human glioblastoma cells. |  (48) |
| Curcumin suppresses glioblastoma cell proliferation by p-AKT/mTOR pathway and increases the PTEN expression. | (58) |
| Anti-Migratory Effect of Vinflunine in Endothelial and Glioblastoma Cells Is Associated with Changes in EB1 C-Terminal Detyrosinated/Tyrosinated Status. | (59) |
| Tubeimoside-1 Inhibits Glioblastoma Growth, Migration, and Invasion via Inducing Ubiquitylation of MET. | (60) |
| Alantolactone, a natural sesquiterpene lactone, has potent antitumor activity against glioblastoma by targeting IKKβ kinase activity and interrupting NF-κB/COX-2-mediated signaling cascades. | (47) |
| Cannabisin D from Sinomenium Acutum Inhibits Proliferation and Migration of Glioblastoma Cells through MAPKs Signaling. | (45) |
| Violacein induces p44/42 mitogen-activated protein kinase-mediated solid tumor cell death and inhibits tumor cell migration. | (61) |
| Xyloketal B Suppresses Glioblastoma Cell Proliferation and Migration in Vitro through Inhibiting TRPM7-Regulated PI3K/Akt and MEK/ERK Signaling Pathways. | (43) |
| JSI-124 Suppresses Invasion and Angiogenesis of Glioblastoma Cells In Vitro. | (62) |
| Coibamide A, a natural lariat depsipeptide, inhibits VEGFA/VEGFR2 expression and suppresses tumor growth in glioblastoma xenografts. | (63) |
| Curzerene suppresses progression of human glioblastoma through inhibition of glutathione S‐transferase A4. | (40) |
| Anti-tumour activity of deer growing antlers and its potential applications in the treatment of malignant gliomas. | (49) |
| Atorvastatin suppresses glioma invasion and migration by reducing microglial MT1-MMP expression. | (11) |
| Simvastatin Inhibits Tumor Growth and Migration by Mediating Caspase-1–Dependent Pyroptosis in Glioblastoma Multiforme. | (64) |
| Apatinib inhibits glioma cell malignancy in patient-derived orthotopic xenograft mouse model by targeting thrombospondin 1/myosin heavy chain 9 axis. | (65) |
| Effect of Blonanserin on the Proliferation and Migration of Glioblastoma Cells. | (50) |
| Fluvoxamine, an anti-depressant, inhibits human glioblastoma invasion by disrupting actin polymerization. | (66) |
| Norepinephrine inhibits migration and invasion of human glioblastoma cell cultures, possibly via MMP-11 inhibition. | (42) |
| Ibuprofen and Diclofenac Restrict Migration and Proliferation of Human Glioma Cells by Distinct Molecular Mechanisms. | (67) |
| Anti-invasive efficacy and survival benefit of the YAP-TEAD inhibitor verteporfin in preclinical glioblastoma models. | (68) |
| Apcin inhibits the growth and invasion of glioblastoma cells and improves glioma sensitivity to temozolomide. | (69) |
| Monensin inhibits glioblastoma angiogenesis via targeting multiple growth factor receptor signaling. | (44) |
| Sulforaphane-cysteine inhibited migration and invasion via enhancing mitophagosome fusion to lysosome in human glioblastoma cells. | (70) |
| Small-molecule agonists of mammalian Diaphanous–related (mDia) formins reveal an effective glioblastoma anti-invasion strategy. | (71) |
| Identification of repaglinide as a therapeutic drug for glioblastoma multiforme. | (51) |

**Appendix Table S3** Quality assessment for in vitro studies (Lewis et al., 2017)

**Remarks:**

1 : Yes

2 : No

3 : Not applicable

Alternative questions:

\* : Assessed based on alternative questions (\*\*, \*\*\*, \*\*\*\*) mentioned below.

\*\* : Or were control available in the study?

\*\*\* : Or were cell lines condition from control and intervention compared?

\*\*\*\* :Or are all results from control and intervention reported?

|  |  |  |
| --- | --- | --- |
| **(Author, publication year)** | **Appraisal Questions** (Lewis et al., 2017) | **Total scores** **out of 6** |
| **Have the cells been obtained from a validated repository that guarantees cell verification or have the cells been appropriately independently verified?** | **Have the experiments been performed in sufficient number of times and were appropriate controls included?** | **Were different cell lines from the same cancer type used in the study? \*\*** | **Are culture conditions comparable between different studies?** | **Were cell lines from different cancer types compared?\*\*\*** | **Are all results from several cell line experiments reported?\*\*\*\*** |
| (Saleh et al., 2019)  | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Rao, Nelson, et al., 2013)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Bagó et al., 2016)  | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Ngo et al., 2020)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Yao et al., 2022)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (C. Wang et al., 2014)\*  | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Rao, DeJesus, et al., 2013)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Fedorova et al., 2023)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Jain et al., 2014)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Lee et al., 2014)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (L.-J. Chen et al., 2021) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (W.-L. Chen, Barszczyk, et al., 2015)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| (Aroui et al., 2020)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
|  |
| (Aroui et al., 2016) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Sun et al., 2022) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Q. Wang et al., 2016) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Guan et al., 2015) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Hei et al., 2019) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Razali et al., 2022)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Z. Wang et al., 2020) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Rovini et al., 2013)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Cao et al., 2019)  | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (X. Wang et al., 2017) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Zhang et al., 2021) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Mehta et al., 2015)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (W.-L. Chen, Turlova, et al., 2015)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Yuan et al., 2015) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Serrill et al., 2016) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Cheng et al., 2022) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Chonco et al., 2021) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Yongjun et al., 2013)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Yang et al., 2022). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Yao et al., 2021).  | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Tsuchiya et al., 2023). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Hayashi et al., 2016). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Zhong et al., 2021). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Leidgens et al., 2015). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Barrette et al., 2022). | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Ding et al., 2021)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Wan et al., 2020) | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Zhou et al., 2020)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |
| (Xiao et al., 2017)\* | 1 | 1 | 1 | 1 | 1 | 1 | 6 |  |

**Appendix Table S4** Quality assessment for in vivo studies (72)

**Remarks:**

6 : Agree strongly

5 : Agree moderately

4 : Agree slightly

3 : Disagree slightly

2 : Disagree moderately

1 : Disagree strongly

CA : I cannot answer

NA : Not applicable

\* : Substance refer to the nanofiber materials

\*\* : No experiment bias score 6; experiment bias occurred score 1

\*\*\* : No variability score 6; variability available score 1

| **Author, publication year** | **Appraisal Questions** (72) | **Total** **out of 78** |
| --- | --- | --- |
| **Substance** | **Experimental animals** | **Assay** | **Measured effects** | **Tested exposure** | **Laboratory procedures and human factors\*\*** | **Result reporting** | **Result analysis** | **Causal intepretation** | **Results intepretation: epistemological context** | **Results check** | **Results interpretation expert judgement** | **Variability****\*\*\*** |
| (Saleh et al., 2019) \* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Grodecki et al., 2015) \* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Kievit et al., 2013) \* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Bagó et al., 2016) \* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Jain et al., 2014) \* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Da Silva et al., 2020) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Aroui et al., 2020) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Sun et al., 2022) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Q. Wang et al., 2016) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Hei et al., 2019) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Z. Wang et al., 2020) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Serrill et al., 2016) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Cheng et al., 2022) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Yao et al., 2021).  | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Hayashi et al., 2016). | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Barrette et al., 2022). | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Wan et al., 2020) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Arden et al., 2015). | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |
| (Xiao et al., 2017). | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 78 |

**Appendix Table S5** Biomimetic techniques and material used as nanofibers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomimetic techniques** | **Materials for biomimetic techniques** | **Type of GBM models** | **Type of cells** | **Development stage** | **(Author, publication year)** |
| Nanofibers to mimic white matter tracts.  | Polyacrylonitrile (PAN)-derived nanofiber (NF) | neurosphere | Gli4 and GliT  | in vitro and in vivo | (Saleh et al, 2019)  |
| Electrospun poly(caprolactone) (PCL) nanofiber scaffolds | Patient-derived OSU-2 Cell Culture | in vivo | (Grodecki et al., 2015)  |
| Aligned chitosan-polycaprolactone polyblend nanofibers (Chitosan-PCL polyblend nanofibers) | Patient-derived OSU-2 cell culture | in vivo | (Kievit et al., 2013)  |
| Core-shell electrospun * Core: gelatin, poly(ether sulfone), poly(dimethylsiloxane))
* Shell: poly(ε-caprolactone) (PCL) material to conserve surface chemistry
 | Patient-derived OSU-2 cell culture | in vitro | (Rao et al., 2013b)  |
| Poly(L-lactic acid) Biocompatible electrospun nanofibrous scaffolds (bENS) or PLA bENS | U87, LN229 and U251 human glioma cell | in vitro and in vivo | (Bagó et al., 2016)  |
| **Total studies: 5\*** |

 **Remarks:**

\*: There were 5 studies using nanofibers as biomimetic techniques

**Appendix Table S6** Biomimetic techniques and material used as hydrogels and other extracellular matrix

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomimetic techniques** | **Materials for biomimetic techniques** | **Type of GBM models** | **Type of cells** | **Development stage** | **(Author, publication year)** |
| Hydrogels to mimic the brain ECM. | Hydrogel from gelatin methacrylamide (GelMA) | neurosphere | U87-MG | in vitro | (Ngo et al., 2020)  |
| Collagen hydrogel | Primary human glioma cells (HCM-BROD-0002-C71, ATCC, Manassas, VA, USA) | in vitro | (Yao et al., 2022)  |
| PEG-based hydrogels | U87 glioma cells | in vitro | (Wang et al., 2014)  |
| Collagen hyaluronan composite hydrogels (from collagen and thiolated HA) | Patient Tumor Derived OSU-2 Cell Culture | In vitro | (Rao et al., 2013a)  |
| Other technique to mimic brain ECM. | GLICO model using poly-HEMA-treated nonadherent | U87-GFP | In vitro | (Fedorova et al., 2023)  |
| **Total studies with hydrogels: 4\*****Total other study: 1\*** |

**Remarks:**

\*: There were 4 studies using hydrogels and 1 other study using GLICO culture as biomimetic technique

**Appendix Table S7**Biomimetic techniques and material used in biomimetic techniques utilizing the combination of biomimetic techniques

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Biomimetic techniques** | **Materials for biomimetic techniques** | **Type of GBM models** | **Type of GBM cells** | **Development stage** | **(Author, publication year)** |
| Combination of nanofibers and hydrogels | * PCL-aligned nanofibers,
* Collagen hydrogel or a cyclopamine-conjugated collagen hydrogel.
 | neurosphere | U87MG-eGFP  | In vitro and in vivo | (Jain et al., 2014)  |
| * Hydrogel with acrylation of hyaluronic acid and microfluidic chip,
* Electrospun nanofiber membrane from polyurethane (PU)
 | glioma cell line A-172 (American Type Culture Collection [ATCC] | In vitro | (Lee et al., 2014)  |
| **Total studies: 2\*** |

**Remarks:**

\*: There were 2 studies using a combination of nanofibers and hydrogels as biomimetic techniques

**Appendix Table S8** Source of natural compounds as potential anti-migratory drugs for GBM treatment

| **Group of natural substances** | **Natural compound name** | **Source of compound** | **Previously identified activities** | **Migration and invasion assays** | **Other supporting assays** | **Type of GBM cells used** | **Development stage** | **Molecular mechanism studied related to GBM migration and invasion** | **(Author, publication year)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Flavonoid | Flavonoid rutin and aglycone quercetine\* | Brazilian plant *Dimorphandra mollis Bent* | Gastroprotective, hepatoprotective, anti-diabetic effects, anti-inflammatory, anti-glycation activities, and reduction of anxiety. | Wound healing and transwell assays | * Cell proliferation assay using Bromodeoxyuridine
* Molecular mechanism assay (ELISA assay for cytokines)
 | U251 and TG1 glioblastoma cells | in vitro and in vivo | * Upregulating the expression of IL-1β, IL-18, CX3CL1, PTGS2 and NOS2
* Downregulating the expression of IL-6, TGFβ, arginase, HDGF, and IGF (Da Silva et al., 2020)
 | (Da Silva et al., 2020) |
| Luteolin and apigenin\* | *Wedelia chinensis* | Anticancer properties effective against prostate, lung, breast, glioblastoma, colon, and pancreatic cancer cells | Transwell migration assay | * Cell apoptosis assay using immunoblotting and
* Autophagy assay using monitoring Autophagy-Immunofluorescence Staining
 | Human malignant glioma cell lines, GBM8401 and U-87MG,and human brain astrocyte (HBA) cells  | in vitro | Not yet studied | (Chen et al., 2021) |
| Carvacrol | Naturally synthesized | Antimicrobial, antibacterial, antiviral, antifungal, and antiprotozoal based on previous studies | Wound healing and transwell assays | * Cell proliferation assay using MTT assay
* Cell apoptosis assay
* Molecular mechanism assay
 | U87 | in vitro | Inhibiting TRPM7 (Chen et al., 2015a) | (Chen et al., 2015a) |
| Naringin(flavanones)\*\* | Citrus fruits | Antioxidant and anti-cancer-related properties | Transwell migration assay | In vitro: * Cell proliferation assay using MTT assay
 | Human malignant glioma cell line, U87 | in vitro and in vivo | * Inducing by VEGF in a dose-dependent manner which caused inhibition of the activation of VEGFR2 after VEGF stimulation,Inhibition of p-AKT and p-ERK caused inhibition of the phosphorylation of p-AKT and p-ERK (Aroui et al., 2020)
* Inhibiting the phosphorylation of JNK1/2, ERK1/2 and p38 pathways (Aroui et al., 2016)
 | (Aroui et al., 2020) |
| In vivo using a xenograft mouse model * Molecular mechanism assays in vivo
 |
| Wound healing assayBoyden chamber | * Cell viability and cell cycle distribution assay
* Molecular mechanism assay
 | U87 and U373 | in vitro | (Aroui et al., 2016) |
| Alkaloid | Berberine | Not mentioned  | Traditional Chinese medicines for bacterial diarrhea | Wound healing assay | In vitro:* Proliferation cell assay
* Molecular mechanism assay
 | Human glioma cells U87 MG, U251, U118 MG, A172, T98G, LN18, LN229, and human umbilical vein endothelial cell (HUVEC) | in vitro and in vivo | Inhibiting the expression of COL11A1 (Sun et al., 2022) | (Sun et al., 2022) |
| In vivo using mouse xenografts:Molecular mechanism assay in vivo |
|  Kukoamine A | Cortex *Lycii radicis* | Traditional Chinese herb and has biological effects for antihypertensive, antioxidant, anti-inflammatory, soybean lipoxygenase inhibition | Wound healing and transwell chamber assay | In vitro:* Proliferation assay using MTT assay
* Cell apoptosis assay using AO/EB staining apoptotic cells
* Cell cycle analysis
 | U251and WJ1 | in vitro and in vivo | Upregulating E-cadherin expression and downregulating C/EBPβ, N-cadherin, vimentin, twist, and snail+ slug expressions (Wang et al., 2016) | (Wang et al., 2016) |
| In vivo: GBM growth in vivo assay |
| Glycosides | Ginsenoside Rh2(steroid glycosides) | Red ginseng | Therapeutic effects on inflammation and a number of cancers | Scratch wound healing assay and transwell cell migration assay | Molecular mechanism assay | The human glioma cell line U251,Patient-derived cell | in vitro | * Inhibiting PI3k/Akt signaling
* Downregulating MMP, MMP2, MMP9, and MMP13
 | (Guan et al., 2015) |
| Verbascoside (phenylethanoid glycoside) | Lemon verbena | Anti-inflammation, antioxidant, and immunomodulation | Transwell chamber | In vitro:Proliferation cell assay | The human GBM cell lines U87, U251 and U87-luciferase | in vitro and in vivo | Reducing c-Met through the proteasome ubiquitination pathway (Hei et al., 2019) | (Hei et al., 2019)  |
| In vivo: U87 xenograft mouse model with VBMolecular mechanism assay in vivo |
| Curcuminoids | Curcumin analogues (FLDP-5 and FLDP-8)\*\*\* | Turmeric | Anti-inflammatory, cytotoxicity, and apoptosis induction on several cancer cell lines | Wound scratch assay | * Proliferation cell assay using MTT assay
* Cell apoptosis assay
* Cell cycle analysis
 | LN-18 human GBM cells. | in vitro | * Inducing cycle termination in U-18 cells to inhibit invasion of GBM cells.
* Inducing oxidative stress through the generation of ROS superoxide anion, which plays an important role in inducing cell apoptosis (Razali et al., 2022)
 | (Razali et al., 2022) |
| Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene3,5-dione)\*\*\* | Turmeric of Curcuma | Adjunctive therapy in some inflammatory and neurodegenerative diseases | Scratch wound healing assays | In vitro:* Cell proliferation assay
* Cell apoptosis assay using The Annexin V-fluorescein isothiocyanate (FITC)-propidium iodide (PI) apoptosis Kit
 | U251and U87 | in vitro and in vivo | * Increasing the expression of PTEN and p53 (Wang et al., 2020)
 |  (Wang et al., 2020) |
| In vivo:Antitumor activity assay |
| Vinca alkaloids  | Vinflunine | Not mentioned  | Approved indication by EMA as treatment of adults with advanced or metastatic urothelial cancers | Wounding-healing assay,transwells | * Cell proliferation assay using MTT assay
* Molecular mechanism assay
 | Human glioblastoma (U87MG) | in vitro | Inhibiting EB1 accumulation (Rovini et al., 2013) | (Rovini et al., 2013) |
| Triterpenoid saponin | Tubeimoside-1 | Rhizoma bolbostemmae | Traditional Chinese medicine as an anti-cancer function in the treatment of esophagus and gastric cancer | Wound-healing assay and Transwells assay | In vitro:* Cell proliferation assay BrdU Staining
* Cell cycle analysis
* Molecular mechanism assay
 | U87 and LN229 cells | in vitro and in vivo | Enhancing the level of ubiquitination of MET (Cao et al., 2019) | (Cao et al., 2019)  |
| In vivo using tumor xenograft |
| Sesquiterpene lactone | Alantolactone | *Inula helenium* | antibacterial, antifungal, anti-inflammatory, and hepatoprotective activities | Wound healing assays and transwell assays | In vitro:* Cell apoptosis assay
* BBB penetration assay (Detection of ATL through the BBB)
* Cell cycle arrest assay
* Molecular mechanism assay
 | U87 and U251 | in vitro and in vivo | Inhibiting COX-2 signaling in human GBM cells | (Wang et al., 2017) |
| In vivo: tumor cell–inoculated miceTumor growth inhibition in vivo |
| Phytocannabinoids | Cannabisin D  | *Sinomenium Acutum* | Traditional Chinese Medicine as treatment for inflammatory and rheumatic diseases | Transwell migration assay | * Cell proliferation assay
* Cell viability assay
* Cell apoptosis assay
* Cell cycle analysis
 | The human glioblastoma cell lines U-251 and U-87 | in vitro | Inhibiting MAPKs Signaling (Zhang et al., 2021) | (Zhang et al., 2021) |
| Hydroxyindoles | Violacein | *Chromobacterium violaceum* | a purple pigment | Boyden chamber assays | * Cell proliferation assay using crystal violet
* Molecular mechanism assay
 | U87 (glioblastoma) | in vitro | Inducing p44/42 mitogen-activated protein kinase (Mehta et al., 2015) | (Mehta et al., 2015) |
| Pentacyclic fungal, dimethyl analogs | Xyloketal B  | Mangrove fungus *Xylaria sp* | Not mentioned  | Wound healing assay | * Cell proliferation assay
* Cell viability assay
* Molecular mechanism assay
 | U251 cells. | in vitro | Suppressing TRPM7 and modulating PI3K/Akt and MEK/ERK signaling pathways (Chen et al., 2015b) | (Chen et al., 2015b) |
| Cucurbitacin | JSI-124 (cucurbitacin I) | Not mentioned | Inhibitor of STAT3 inhibitor and induce the apoptosis of human tumor cell lines | Transwell migration | In vitro:* Cell apoptosis assay
* Cell viability assay
* Molecular mechanism assay
 | U251 and U87MG | in vitro and in vivo | Inhibiting VEGF secretion (Yuan et al., 2015) | (Yuan et al., 2015) |
| In vivo using tumor xenograft model:Molecular mechanism assay |
| Cyclodepsipeptide | Coibamide A | Marine cyanobacteria | Inhibitor growth of cancer cell lines | Transwell migration assays | In vitro:* Cell proliferation assay
* Molecular mechanism assay
 | U87-MG glioblastoma Human SF-295 glioblastoma cells. | in vitro and in vivo | Inhibiting VEGFA/VEGFR2 expression and suppressing tumor growth in glioblastoma xenografts (Serrill et al., 2016) | (Serrill et al., 2016) |
| In vivo using U87-MG GBM xenograft model in mice:Anti-tumor assay  |
| Terpenoid | Curzerene | *Curcuma longa* | Traditional Chinese medicine as anticancer agents | Wound healing assay and transwell migration assay | In vitro:* Cell proliferation assay
* Cell apoptosis assay
* Molecular mechanism assay
 | U251 and U87 | in vitro and in vivo | Inhibiting the activation of the mTOR pathway and the expression of MMP9 (Cheng et al., 2022) | (Cheng et al., 2022) |
| In vivo using tumor xenograft growth in nude mice: Observation of the survival time of tumor-bearing mice  |
| Nucleosides | Deer antler velvet (DAV) extract | Red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), white-tailed deer (*Odocoileus virginianus*), and elk (*Cervus canadensis*) | Anti-cancer | Scratch assay. | * Cell proliferation assay
* Cell apoptosis assay
* Cell viability assay
* Cell cycle analysis
 | T98G and A172 | in vitro | Not yet studied | (Chonco et al., 2021) |
| **Total studies: 21** **Total compounds: 20 natural compounds** |

**Remarks:**

\* : count as one compound because they came from the same source and were tested together

\*\* : count as one compound which had two different studies

\*\*\* : count as two compounds because one study used curcumin and the other one used analogs of curcuminoid

**Appendix Table S9** Potential anti-migratory compound for GBM treatment from small molecules and biologic

| **Type of compound** | **Compound name** | **Repurposed medicines or not** | **Previous approved Indication** | **Migration assay** | **Other supporting assays** | **Type of GBM cells** | **Development Stage** | **Molecular mechanism****related to GBM migration and invasion** | **(Author, year)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Small molecules | Atorvastatin | Yes | Dyslipidaemia treatment acting as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase | Transwell assay | * Cell viability assay
* Molecular mechanism assay
 | A human U87 human primary glioblastoma cell line | In vitro  | * Reducing microglial MT1-MMP expression (Yongjun et al., 2013)
* Inhibiting the phosphorylation status of JNK in U87 GBM cells. (Yongjun et al., 2013)
 | (Yongjun et al., 2013). |
| Simvastatin | Yes | Dyslipidaemia treatment acting as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase | Wound-Healing assay, Transwell Assay | * Cell viability assay
* Cell cycle analysis
* Molecular mechanism assay
 | U87 and U251 human glioma cell lines w | In vitro | Inhibiting Caspase-1 (Yang et al., 2022) | (Yang et al., 2022). |
| Apatinib | Yes | Non-small cell lung cancer (NSCLC) treatment | Scratch test and transwell assay | In vitro:* Cell proliferation assay
* Molecular mechanism assay
 | Cell culture Two primary human GBM cell lines (N14042 and N14069) were obtained from two GBM patients and the commercial U87 cell line. | In vivo and in vitro | Targeting thrombospondin 1/myosin heavy chain 9 axis (Yao et al., 2021).  | (Yao et al., 2021).  |
| In vivo using patient-derived orthotopic xenograft (PDOX) glioma mouse model:Progression of GBM cells in vivo assay |
| Blonanserin | Yes | Treatment of schizophrenia. | Scratch assay | * Cell proliferation assay
* Cell viability assay
* Cell growth inhibitory assay
 | Human glioblastoma cell lines (U251 and T98G cells) | In vitro | Not yet studied | (Tsuchiya et al., 2023). |
| Fluvoxamine | Yes | Antidepressant | Wound-healing assayMatrigel invasion assay | In vitro:* Cell proliferation assay using WST-1 assay
* Actin polymerization assay
* In vitro actin assembly assay.
* Molecular mechanism assay
 | Human GBM cell lines U87-MG, U251-MG, and A172 | In vitro and in vivo | Inhibiting FAK and Akt/mTOR signaling. (Hayashi et al., 2016) | (Hayashi et al., 2016). |
| In vivo using GBM xenograft:Cell proliferation assay  |
| Norepinephrine | Yes | Blood pressure control in certain acute hypotensive states | Wound Healing AssayMatrigel or transwell invasion assay | Molecular mechanism assay | Malignant brain tumor cell lines U251 and U87 | In vitro | Downregulating MMP-11 expression (Zhong et al., 2021) | (Zhong et al., 2021). |
| Ibuprofen and Diclofenac\* | Yes | Inflammatory diseases, rheumatoid disorders, and mild to moderate pain | Spheroid migration assays | * Cell proliferation assay
* Cell cycle analysis
* Molecular mechanism assay
 | Human high-grade glioma cell lines U87MG and A172 | In vitro | Decreasing STAT-3 phosphorylation and decreasing c-myc expression (Leidgens et al., 2015) | (Leidgens et al., 2015)**.** |
| Verteporfin (VP) | Yes | Macular degeneration treatment as an inhibitor of YAP-TEAD19 and TAZTEAD | Spheroid dispersion/migration assay and transwell invasion assays | In vitro:* Cell proliferation assay
* Cell viability assay
* Molecular mechanism assay
 | 8 patient-derived lines at low passage (<30) (G-13063, G-12746, G-13181, G-16302, G-13514, G-17969, G-11849, G-11849R) and U87 GBM cell line | In vitro and in vivo | Inhibiting YAP, TEAD1, and TEAD1-target expression in PDX tumors (Barrette et al., 2022) | (Barrette et al., 2022). |
| In vivo using Patient-derived orthotopic xenograft (PDX):Cell proliferation assay |
| Apcin | N/A | N/A | Wound healing assay | * Cell apoptosis assay
* Cell viability assay
* Molecular mechanism assay
 | U251MG | In vitro | Increasing Bim expression in GBM cells after being treated with apcin (Ding et al., 2021) | (Ding et al., 2021). |
| Monensin | N/A | N/A | Transwell assay | In vitro* Cell proliferation assay
* Cell apoptosis assay
* Cell adhesion assay
* Cell growth and survival
* Molecular mechanism assay
 | Human glioblastoma cell lines, U87, U251MG, T98G, A172, U251 N and U373 and HCC287 | in vitro and in vivo | Inhibiting VEGR and EGFR signaling pathways in endothelial cells | (Wan et al., 2020) |
| In vivo:Inhibition angiogenesis and growth of GBM in vivo |
| Sulforaphane-cysteine (SFN-Cys) | N/A | N/A | Wound scratch healing assay,transwell chamber assay | * Autophagy and mitophagy assay
* Molecular mechanism assay
 | Human GBM U87MG | In vitro | Inhibiting Claudin-5 and S100A4 (Zhou et al., 2020) | (Zhou et al., 2020). |
| Small-molecule agonists of mammalian Diaphanous–related (mDia) or mDia agonism | N/A | N/A | Scratch assay, fluoroblock transwell chamber assay | Ex vivo:Spheroid invasion in an ex vivo brain-slice invasion model | U87, U343, U118, T98G, SNF19, SF295, LN229, and A172 | ex vivo | Enhancing F-actin dynamics (Arden et al., 2015) | (Arden et al., 2015). |
| Biologics | Repaglinide | Yes | Oral insulin secretagogue for patients with type 2 diabetes mellitus | Scratch-wound culture model and cell migration assay | In vitro:* Cell proliferation assay using MTT assay
* Cell viability assay
 | Human GBM cells (LN229). | In vitro and in vivo | Not yet studied, however the discussion mentioned that the probability of repaglinide could inhibit GBM cell migration via downregulating Blc-1, Beclin-1, and PD-L1. | (Xiao et al., 2017). |
| In vivo using orthotopic GBM model:* Observation median survival time
* Molecular mechanism
 |
| **Total studies: 13** **Total compounds: 13 (12 small molecules and 1 biologic)**  |

**Remarks:**

\* : count as a single compound combination because they were tested together in the same study