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Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the RAS

Institute of Chemical Biology and Fundamental Medicine
Siberian Branch of the Russian Academy of Sciences

SEMISYNTHETIC TRITERPENOIDS AS PROMISING BLOCKERS OF AGGRESSIVENESS-RELATED TRAITS IN GLIOBLASTOMA MULTIFORME: *IN SILICO*, *IN VITRO*, AND *IN VIVO* APPROACHES

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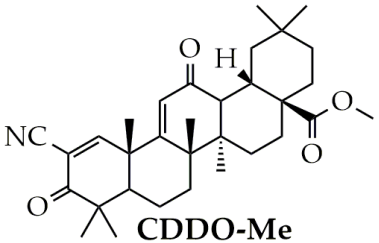
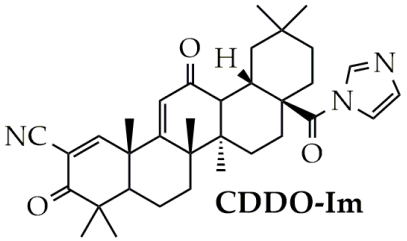
Moscow,
2023

Cyano enone-bearing triterpenoids: high bioactivity, multitargeting, clinical trials

Triterpenoids developed by group of prof. Michael Sporn (Geisel School of Medicine at Dartmouth, USA). Clinical trials by Reata Pharmaceuticals, Inc.



CDDO-Me
(Bardoxolone methyl)



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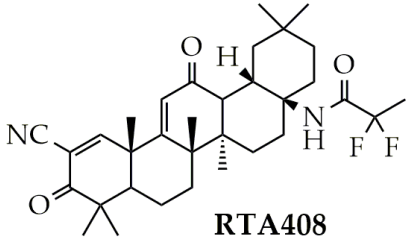
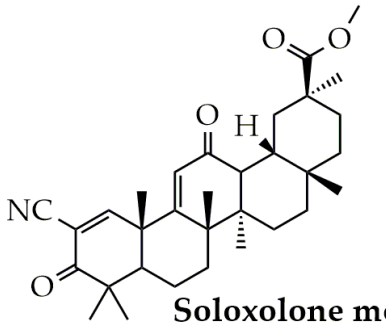
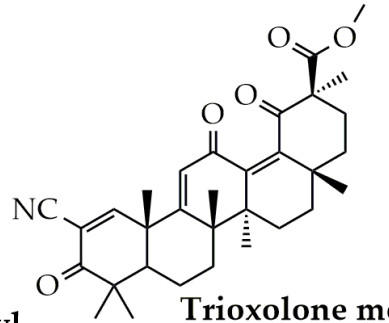


RTA408

Glycyrrhetinic acid-based analogs of CDDO-Me synthesized in the Laboratory of pharmacologically active compounds, NIOCH SB RAS



Soloxolone methyl
(SM)



Trioxolone methyl
(TM)

- Induce of apoptosis of tumor cells (*Logashenko et al., ChemBioChem, 2011*);
- Suppress tumor growth *in vivo* (*Markov et al. Mol. Biol., 2018*);
- Trigger ER stress (*Markov et al. Oncotarget, 2019*);
- Inhibit acute inflammation *in vivo* (*Markov et al. Mol. Biol., 2018*);
- Inhibit peritonitis progression in mice (*Markov et al., Int. J. Mol. Sci., 2020*);
- Ameliorate DSS-induced colitis in mice (*Markov et al., Molecules, 2020*)
- Inhibit acute lung injury in mice (*Sen'kova et al. PLOS One, 2021*)

Clinical trials:

- solid tumors and lymphoid malignancies (Phase 1);
- chronic kidney disease (Phase 3);
- pulmonary hypertension (Phase 3);
- Alport Syndrome (Phase 2/3);
- COVID-19 (Phase 2/3)

Are these compounds able to block the aggressiveness of glioblastoma multiforme?

The aim of the study

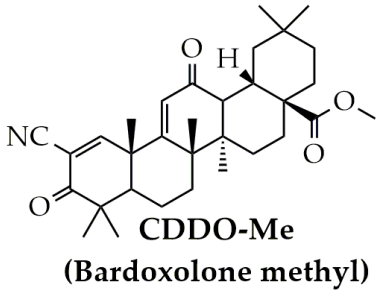
To determine whether cyanoenone-containing pentacyclic triterpenoids can block glioblastoma multiforme aggressiveness

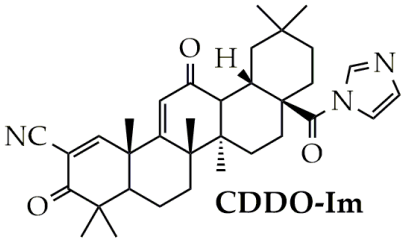
Main tasks:

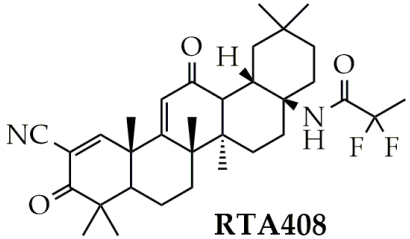
- Synthesis of novel amides of soloxolone
- Evaluation of permeability of novel soloxolone amides through blood-brain barrier
- Evaluation of cytotoxicity of novel compounds against glioblastoma cells
- The study of mechanisms underlying the cytotoxic activity of hit compound
- Exploration of the effect of hit compound on pro-metastatic characteristics of glioblastoma cells
- Evaluation of the effect of novel soloxolone amides on glial-mesenchymal transition in glioblastoma cells
- Verification of anti-glioblastoma potency of hit compound in animal model

Cyano enone-bearing triterpenoids: high bioactivity, multitargeting, clinical trials

Triterpenoids developed by group of prof. Michael Sporn (Geisel School of Medicine at Dartmouth, USA). Clinical trials by Reata Pharmaceuticals, Inc.







Clinical trials:

- solid tumors and lymphoid malignancies (Phase 1);
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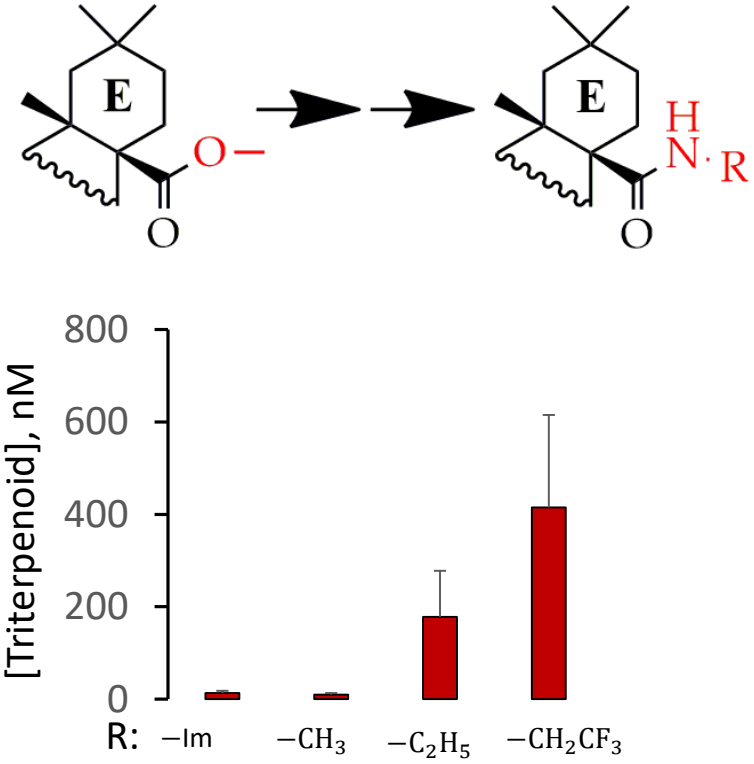
- pulmonary hypertension (Phase 3);
- Alport Syndrome (Phase 2/3);
- COVID-19 (Phase 2/3)

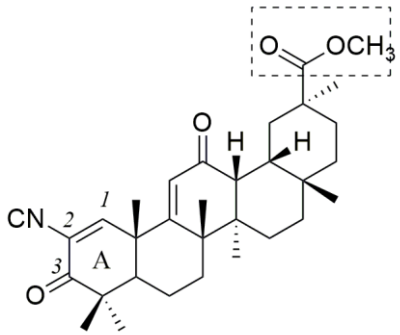
Formation of an amide group at position C-29 enhances the accumulation efficiency of cyano enone-containing triterpenoids in mouse brain



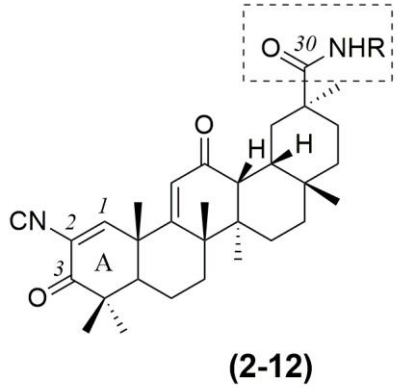
Sporn et al. US patent No. 12/151,425, 2009

Are these compounds able to block the aggressiveness of glioblastoma multiforme?

Novel soloxolone amides. Cytotoxicity



Soloxolone methyl (SM)



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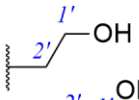
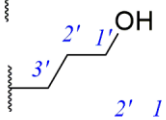
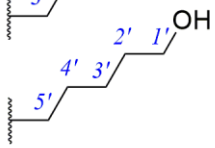
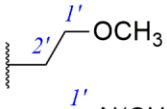
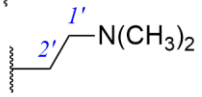
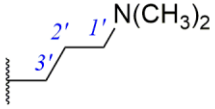
2: R= 

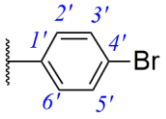
3: R= 

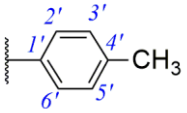
4: R= 

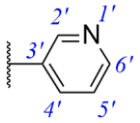
5: R= 

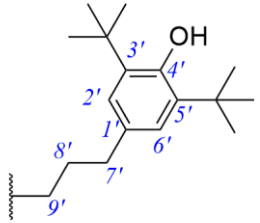
6: R= 

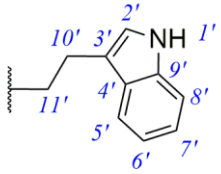
7: R= 

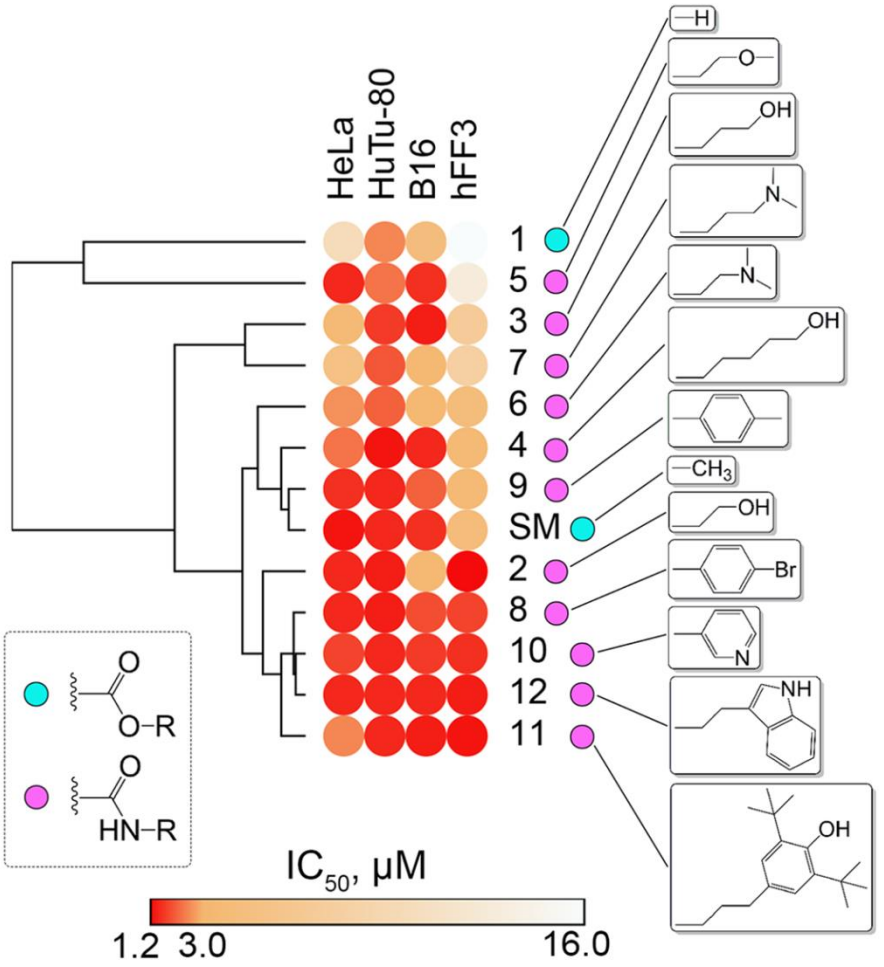
8: R= 

9: R= 

10: R= 

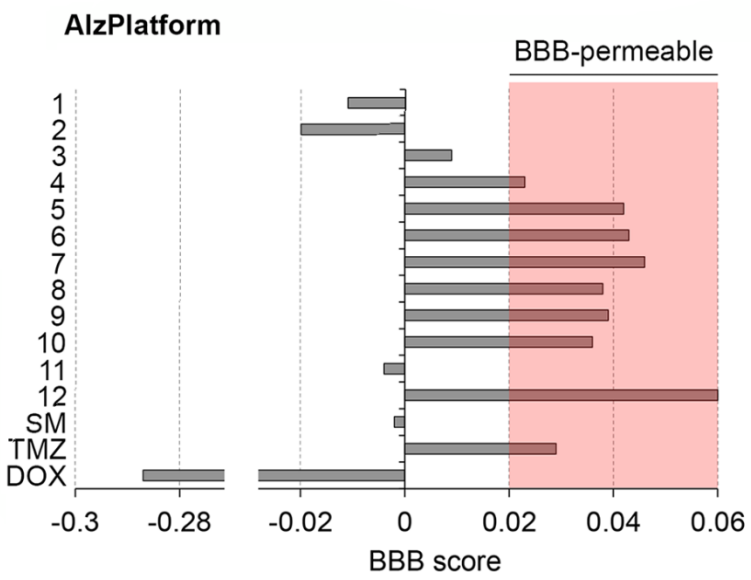
11 : R= 

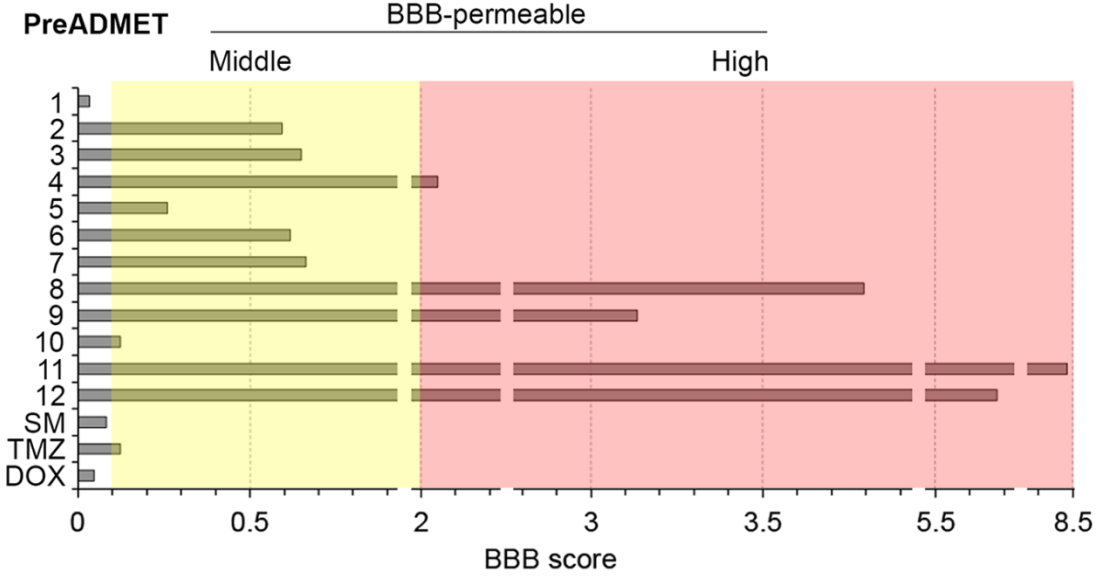
12 : R= 

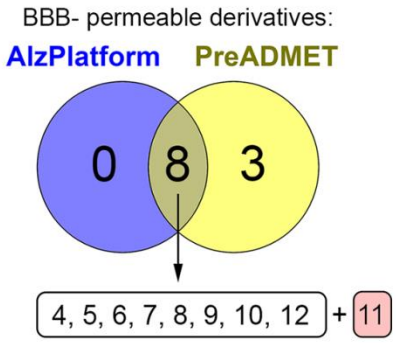


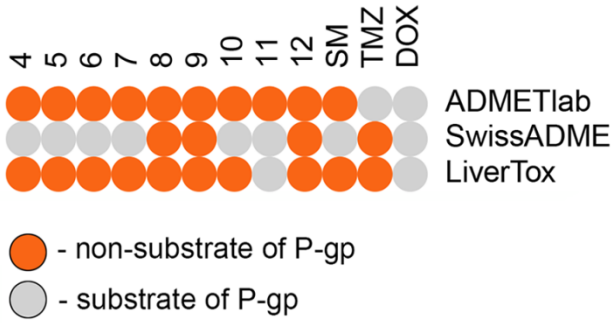
HeLa – human cervical carcinoma;
HuTu-80 – human duodenal adenocarcinoma;
B16 – murine melanoma;
hFF3 – non-malignant fibroblasts

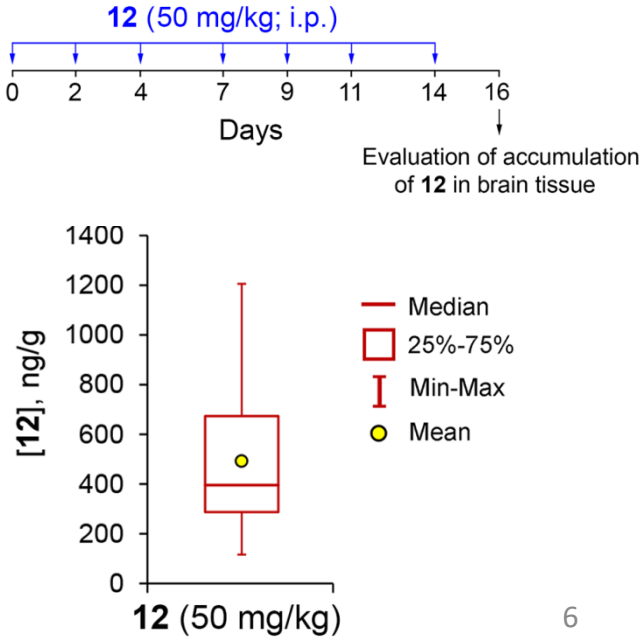
Soloxolone amides are capable to cross the blood-brain barrier







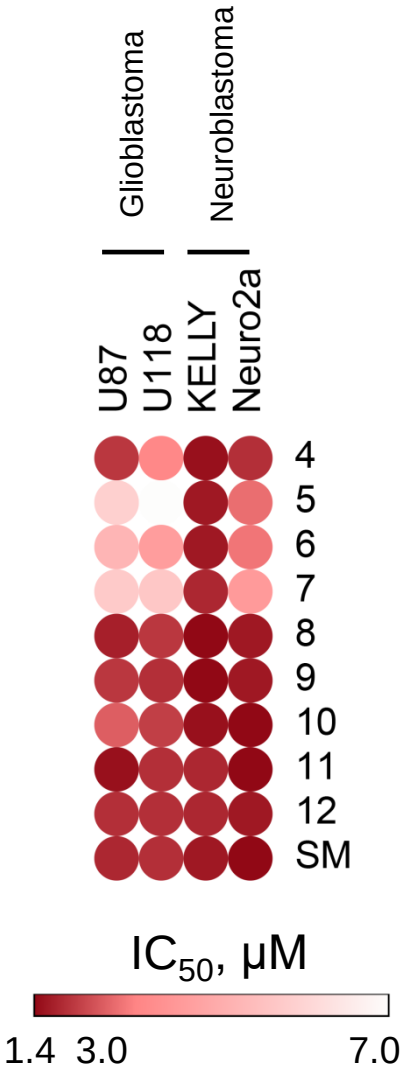




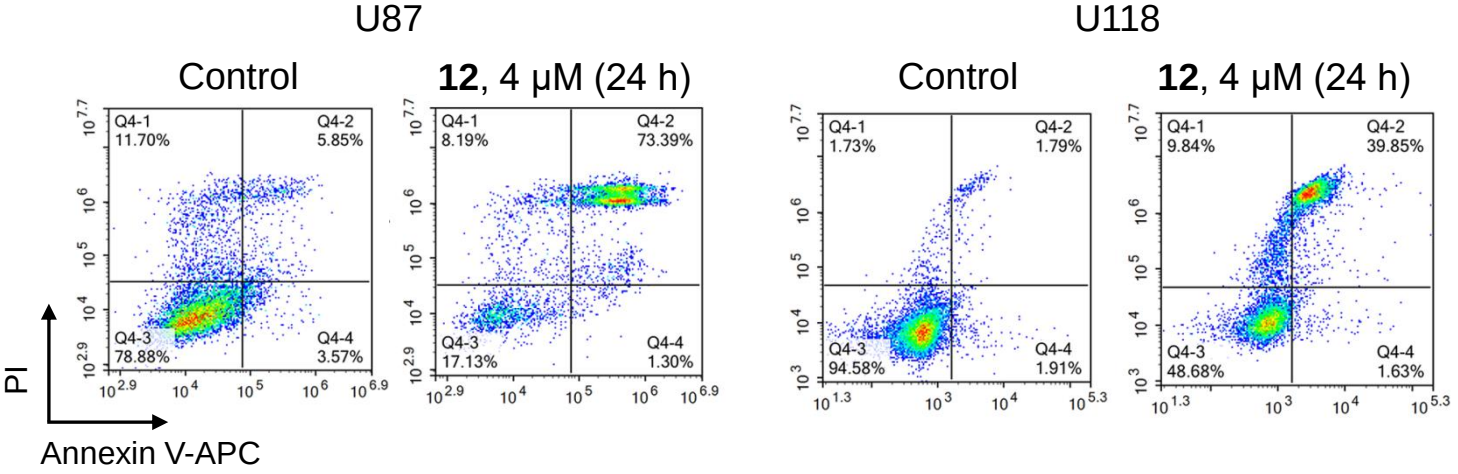
The content of compound **12** in mouse brain tissue was determined by HPLC-MS/MS

Novel soloxolone amides trigger apoptosis in glioblastoma cells

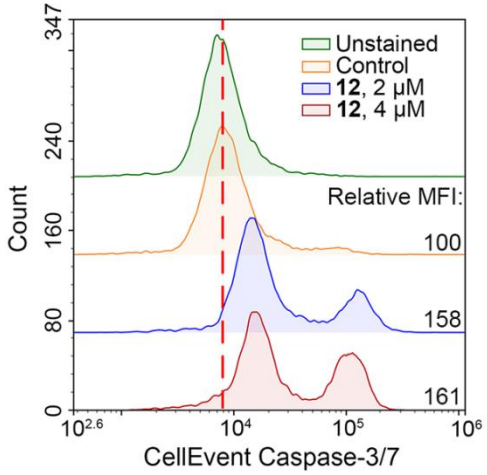
Cytotoxicity of compounds against tumor cells of glial and neuronal origin



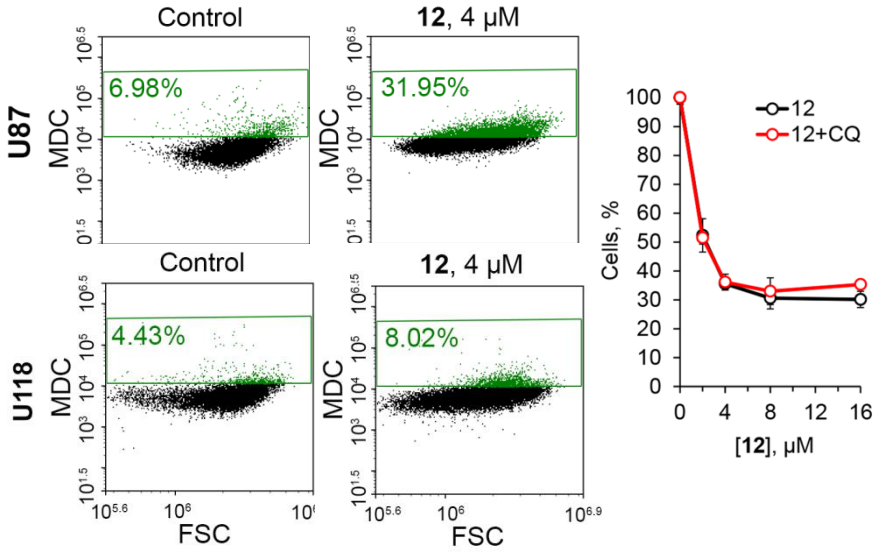
***12** induces phosphatidylserine externalization*



***12** activates caspases-3/-7*

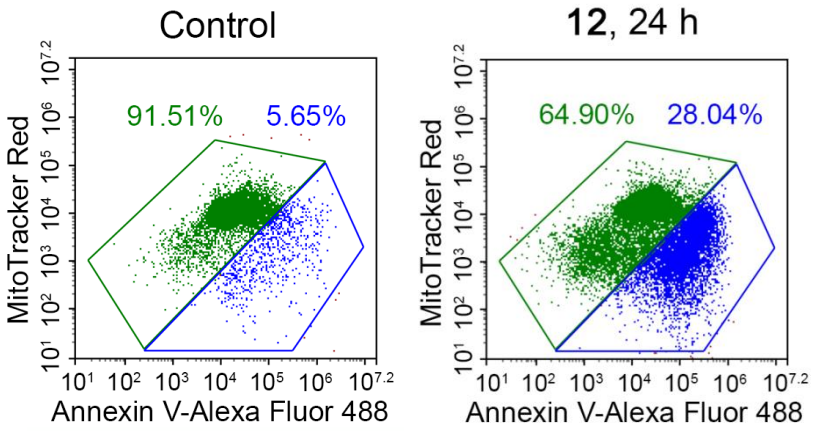


***12** triggers autophagy in U87 cells*

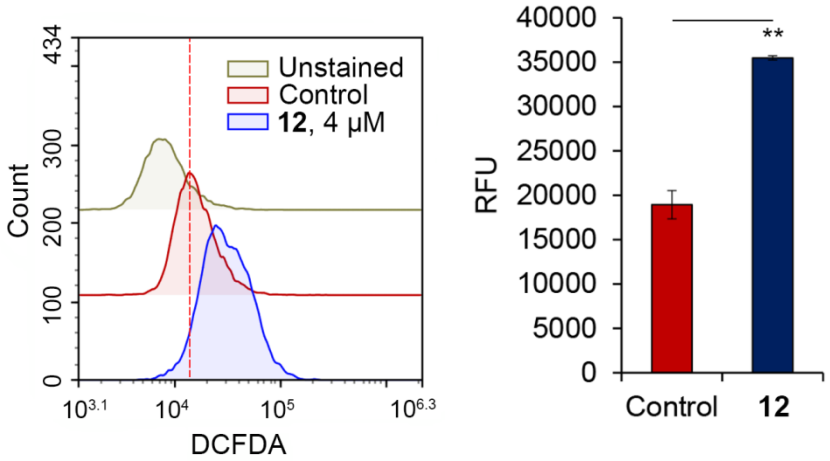


Compound 12 activates mitochondrial pathway of apoptosis in glioblastoma cells

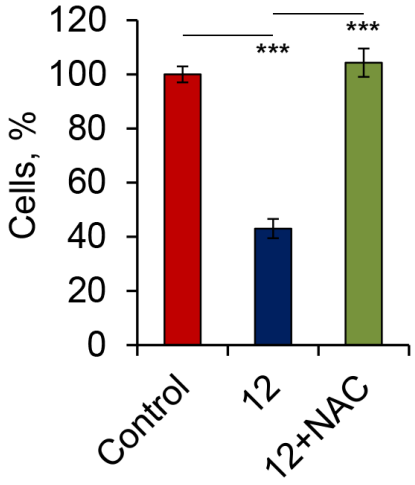
12 *triggers dissipation of mitochondrial membrane potential of U87 cells*



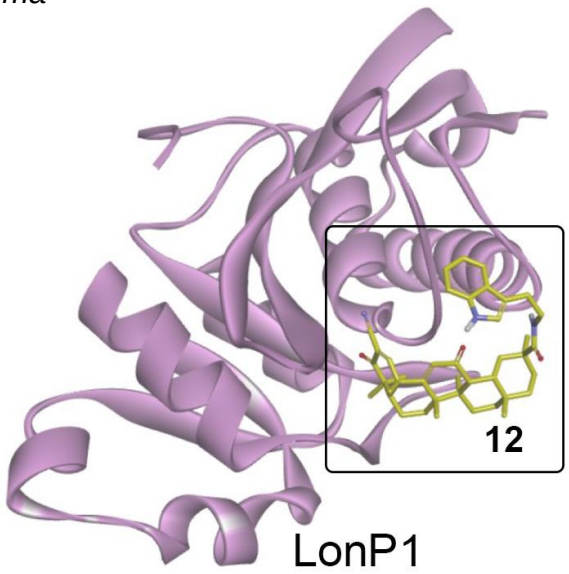
12 *stimulates ROS generation in U87 cells*

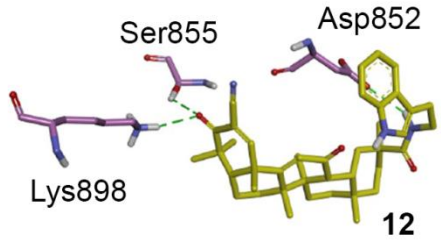


12 *induced ROS-dependent death of glioblastoma cells*



Mitochondrial LonP1 protease is probable primary protein target of ***12*** *associated with its cytotoxicity*

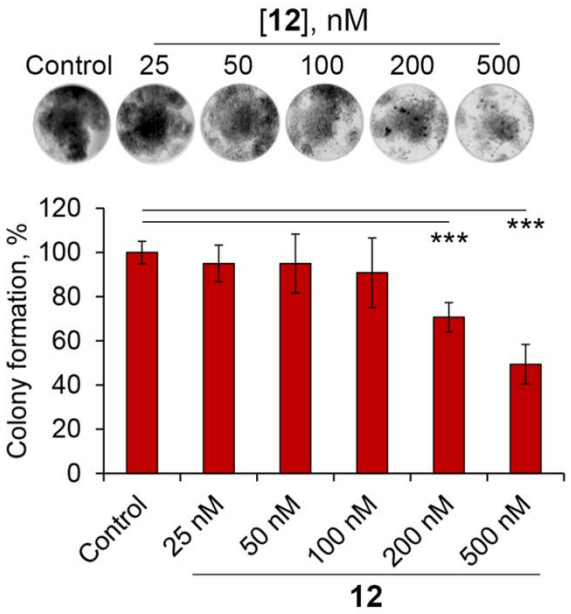




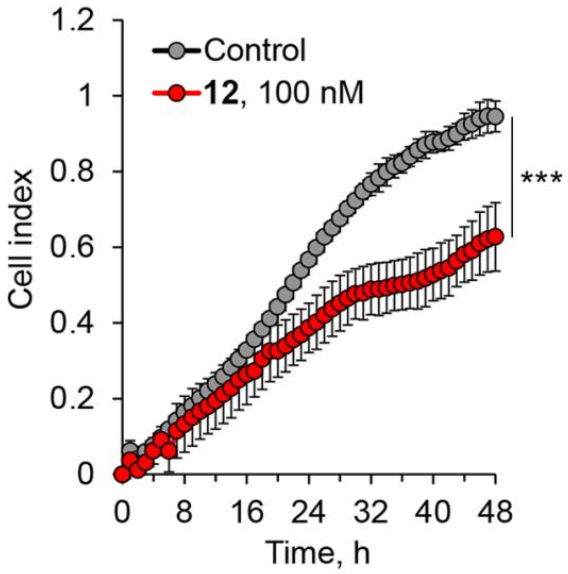
ΔG = -8.8 kcal/mol

Compound 12 demonstrates high anti-glioblastoma potency at non-toxic concentrations

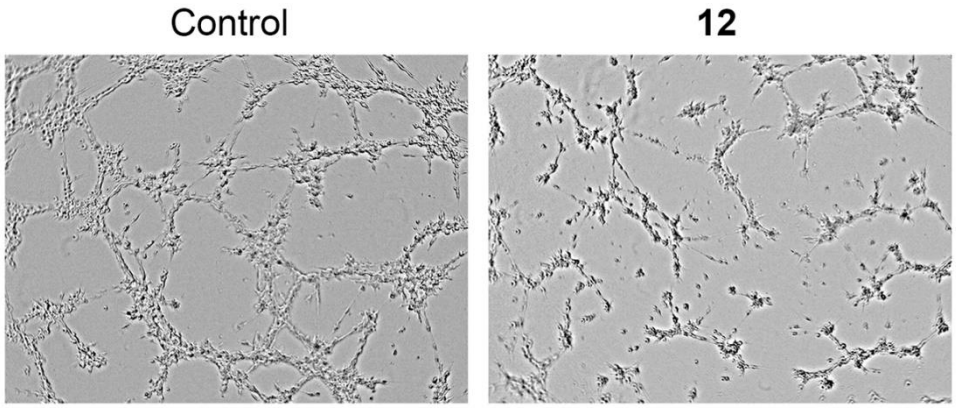
12 *inhibits clonogenicity of U118 cells*

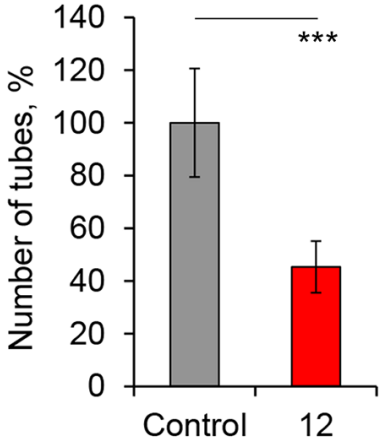


12 *inhibits motility of U118 cells*

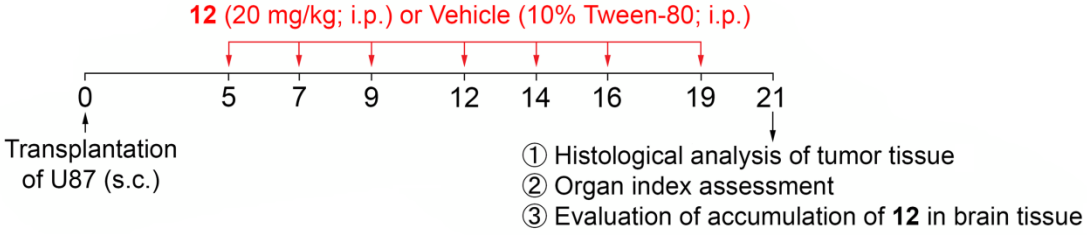


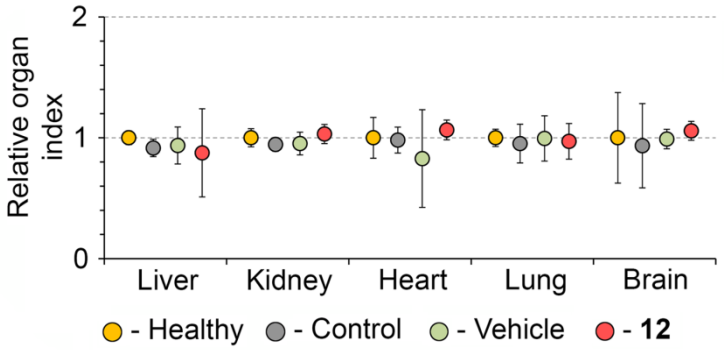
12 *suppresses glioblastoma vasculogenic mimicry (U87 cells)*

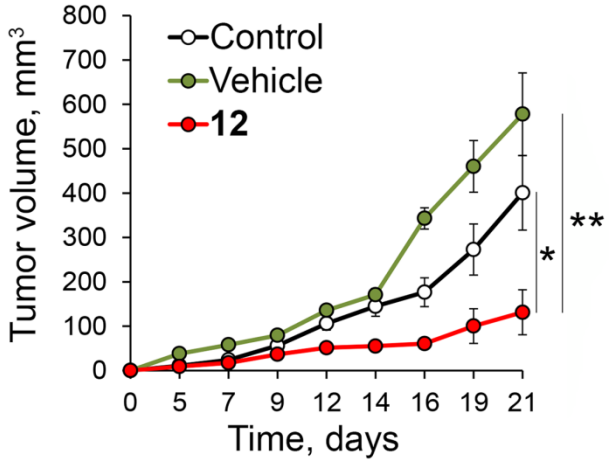


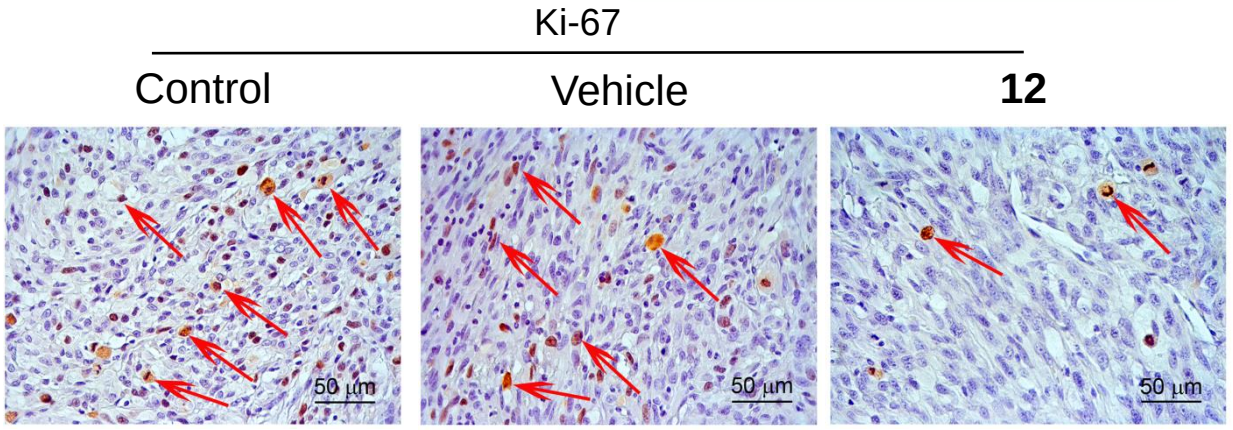


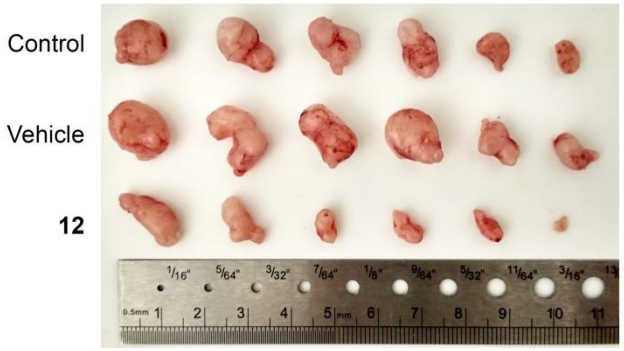
Compound 12 effectively blocks growth of U87 glioblastoma in xenograft model *in vivo*

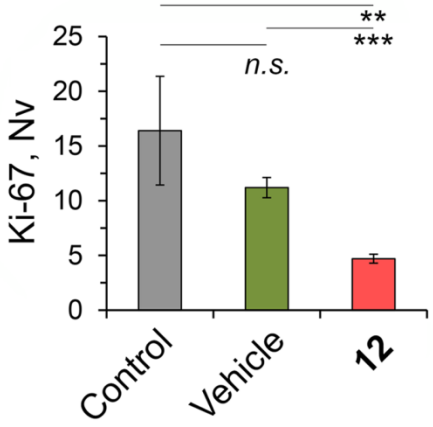


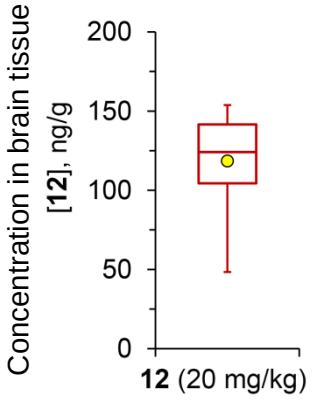












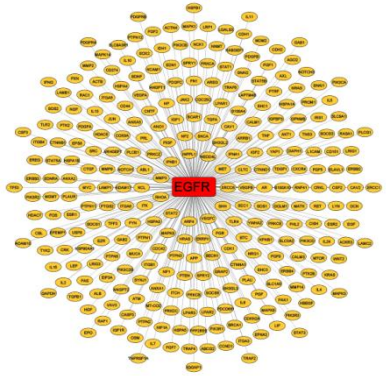
EGFR, AKT1, and ERBB2 are probable primary targets of compound 12 associated with its anti-glioblastoma potency

Probable target prediction:

Polypharmacology browser 2.0

SwissTargetPrediction

Reconstruction of gene association networks

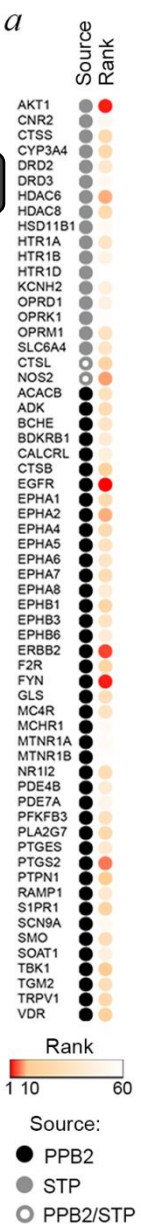


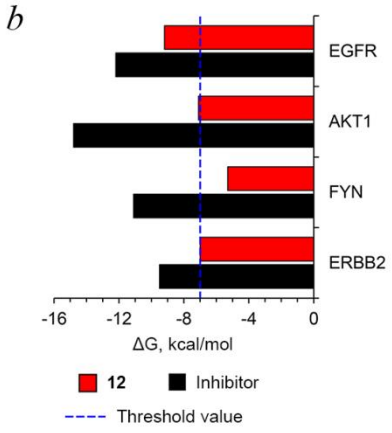
GBM pathogenesis
DisGeNET ID: C1621958

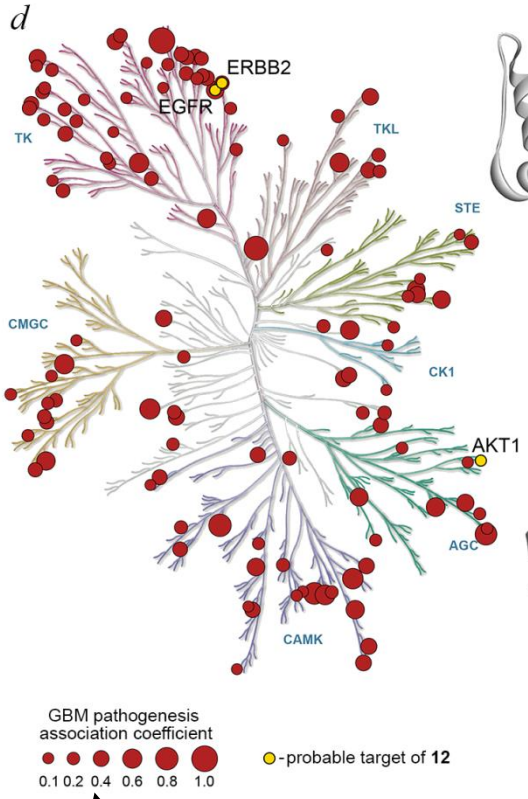
Calculation of target's centrality score:

- Degree
- MCC
- Betweenness

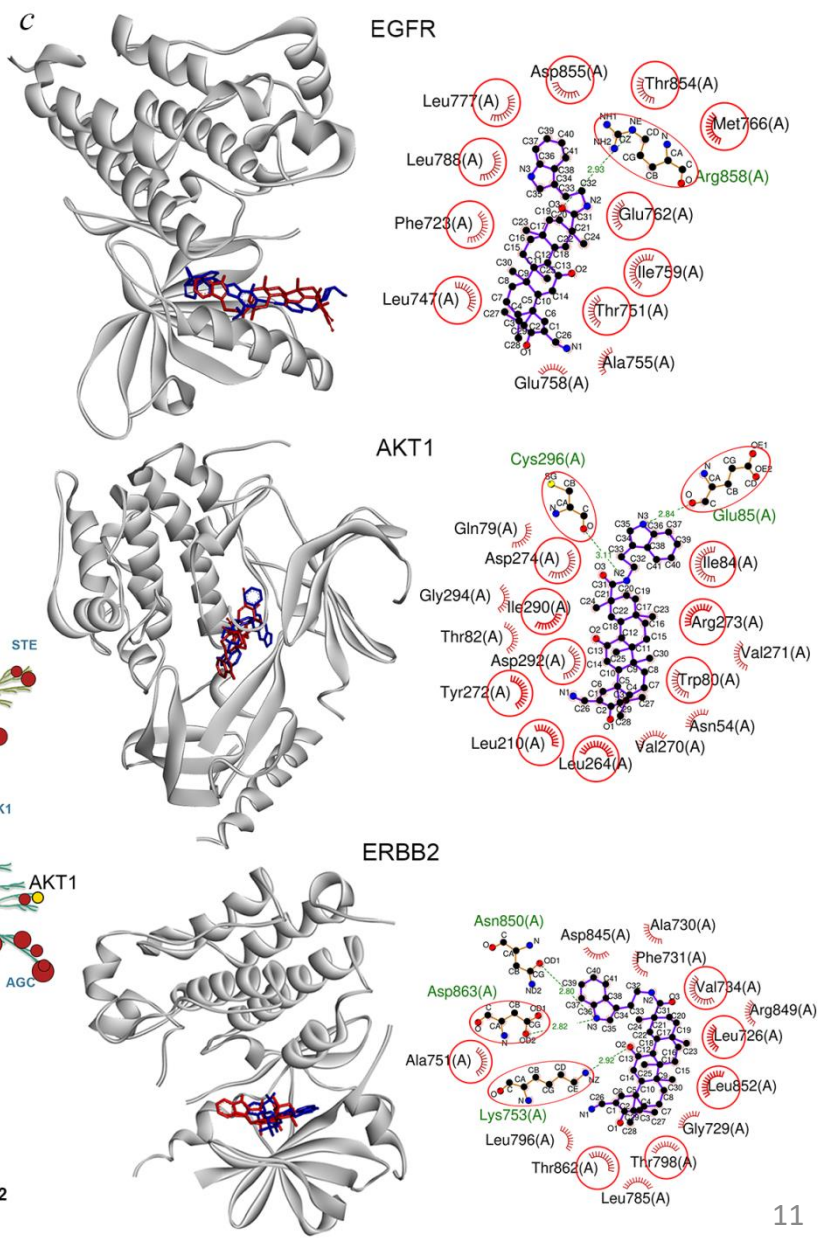
Ranking





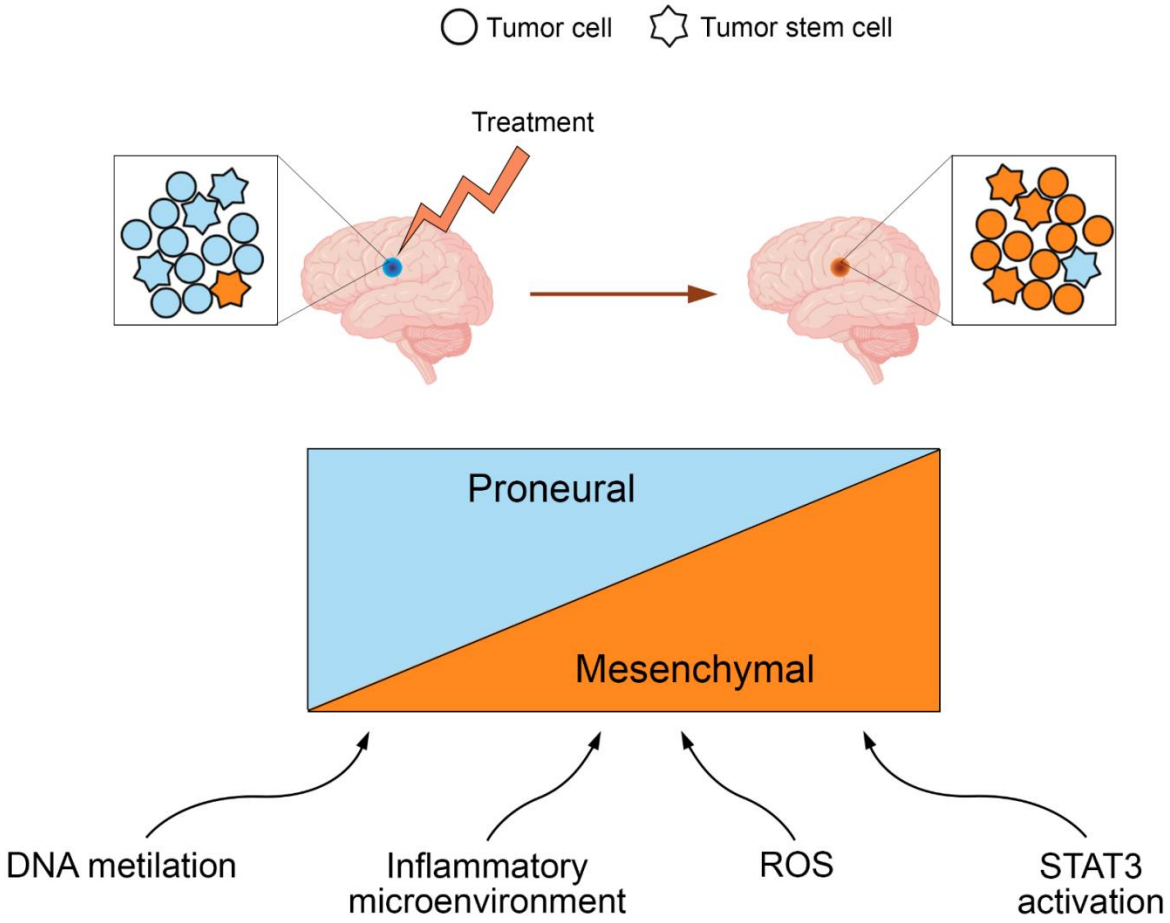


OpenTargets platform data



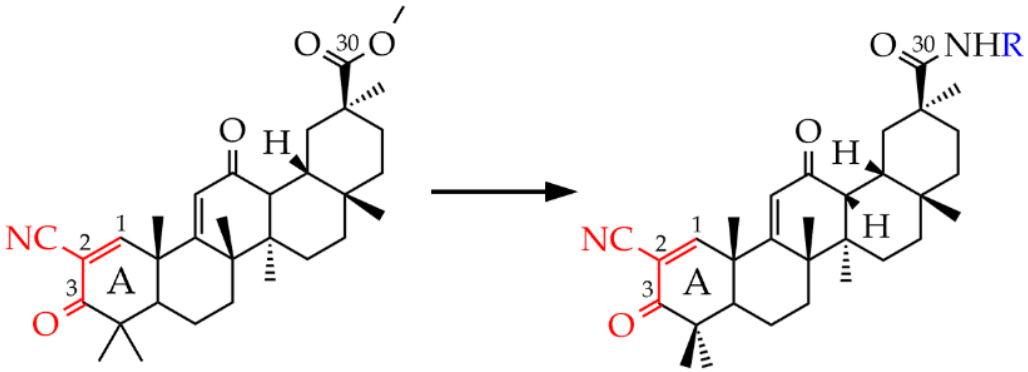
Glial-mesenchymal transition is a key process underlying high aggressiveness of GBM

- There are 4 molecular subtypes of GBM: classical, neuronal, proneural, and mesenchymal. Mesenchymal is the most malignant and is associated with the worst prognosis.
- A shift toward a mesenchymal phenotype, or **glial-mesenchymal transition (GMT)**, occurs during glioblastoma progression due to accumulated mutations and tumor microenvironment factors.
- The glial stem cell population is highly resistant to radio- and chemotherapy - these cells restore tumor growth, giving rise to recurrence.



Fedele et al., 2019; Setlai et al., 2022

Soloxolone amides capable of crossing the blood-brain barrier

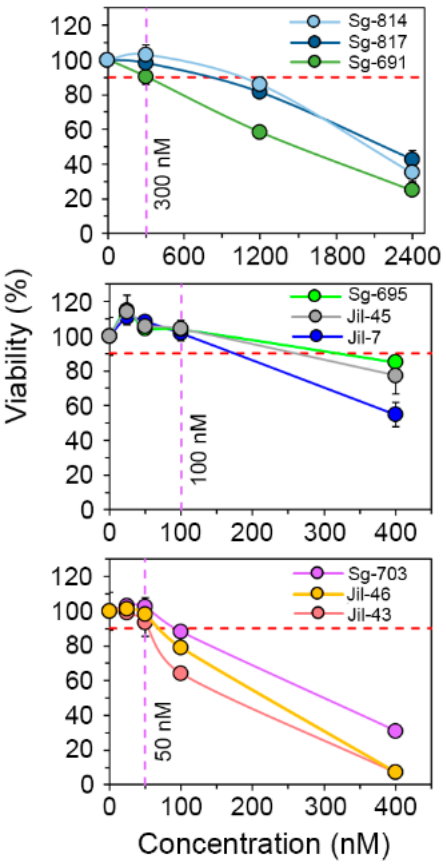


Soloxolone methyl

Soloxolone amides

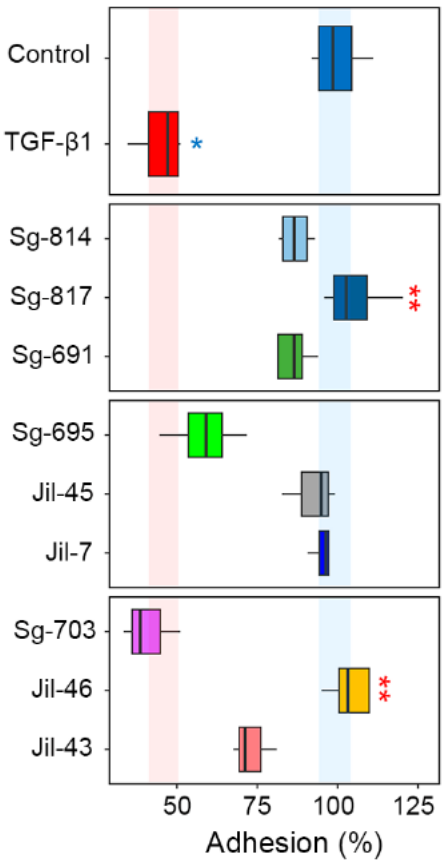
Soloxolone amides effectively suppress processes associated with TGF-β1-induced GMT in U87 cells

Identification of non-toxic concentrations



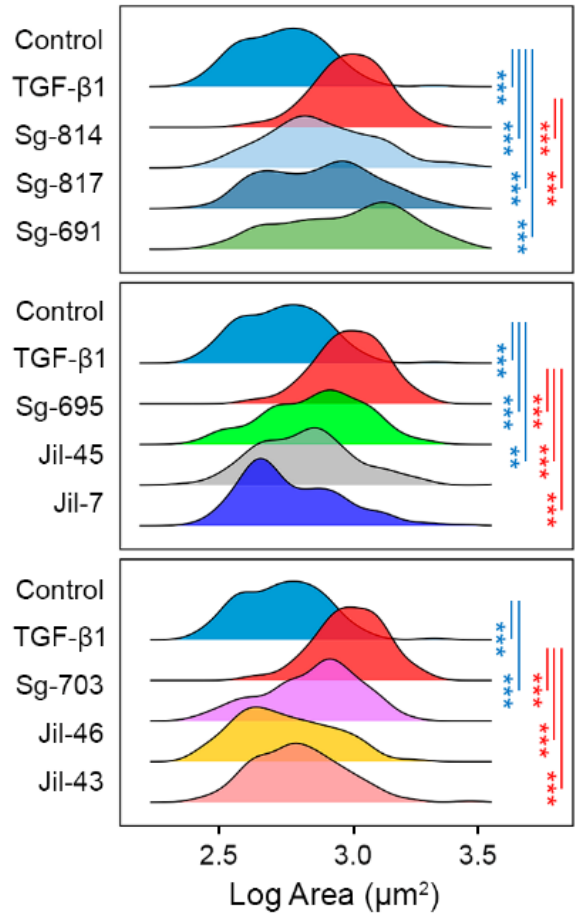
MTT assay data (48 h)

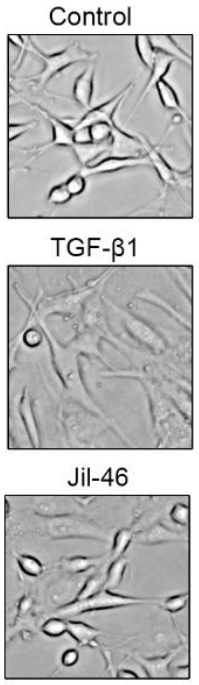
The effect of compounds on cell adhesion



Trypsin test (48 h)

The effect of compounds on cell morphology



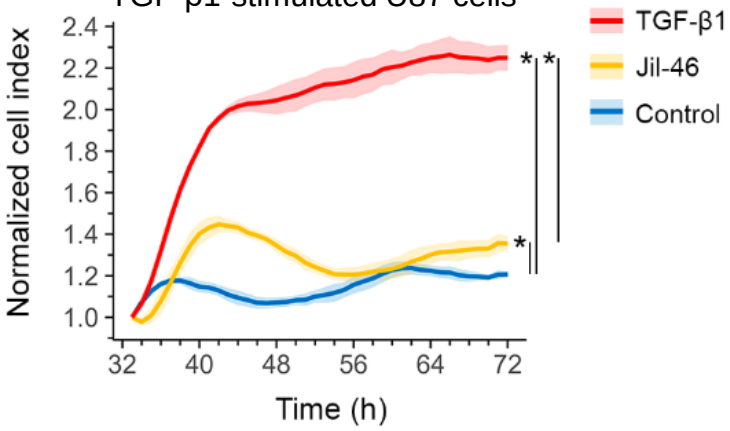


* - p-value < 0.05, ** - p-value < 0.01,
*** - p-value < 0.001

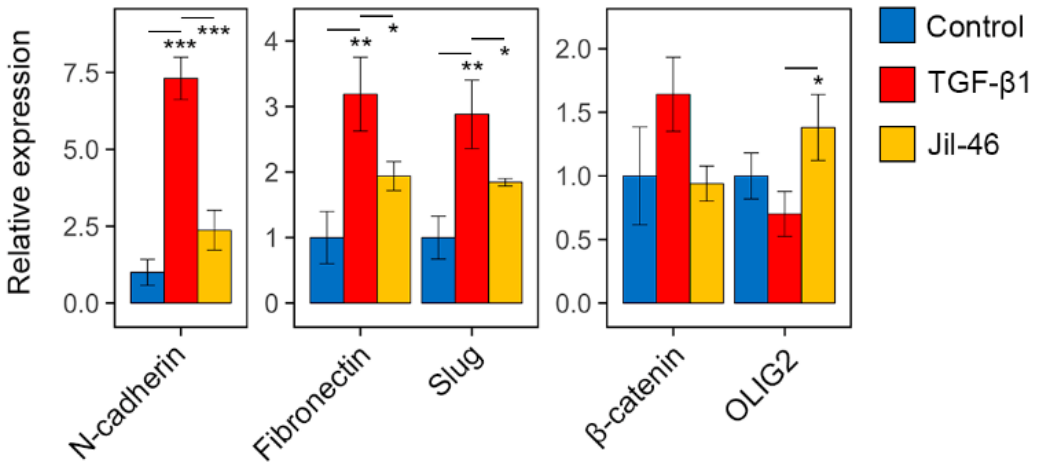
Hit compound: Jil-46

Jil-46 blocks TGF-β1-induced GMT in U87 cells

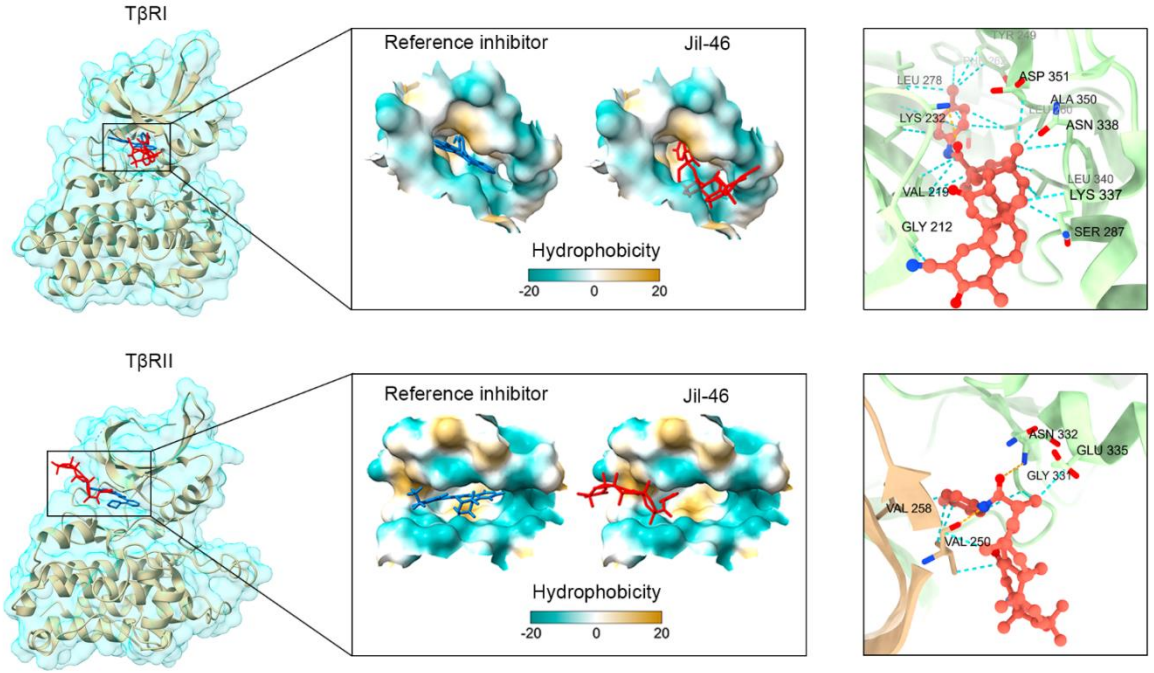
Jil-46 inhibits transwell-migration of TGF-β1-stimulated U87 cells

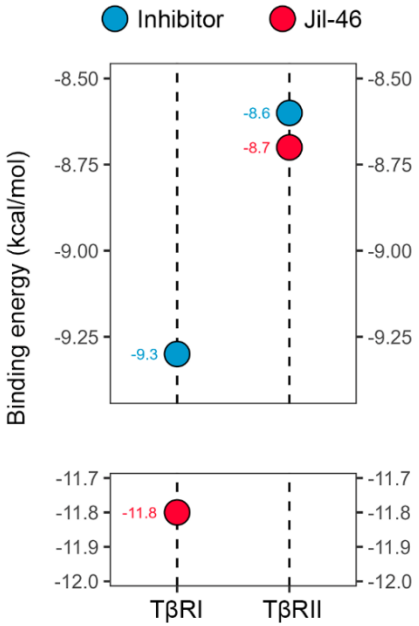


The effect of Jil-46 on key markers of GMT

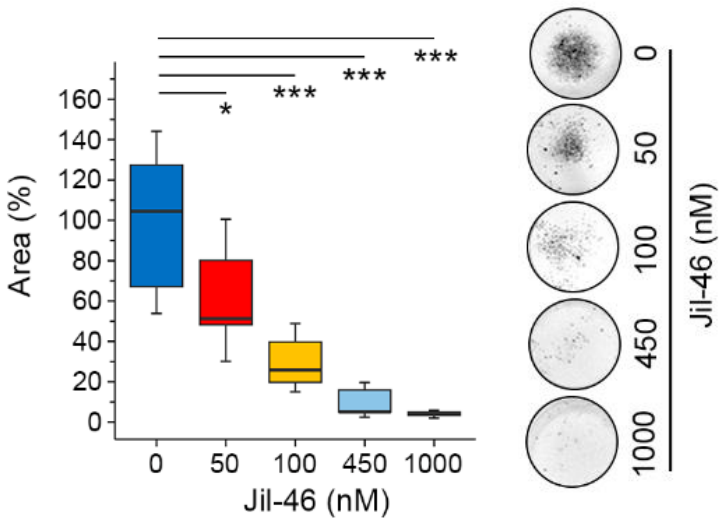


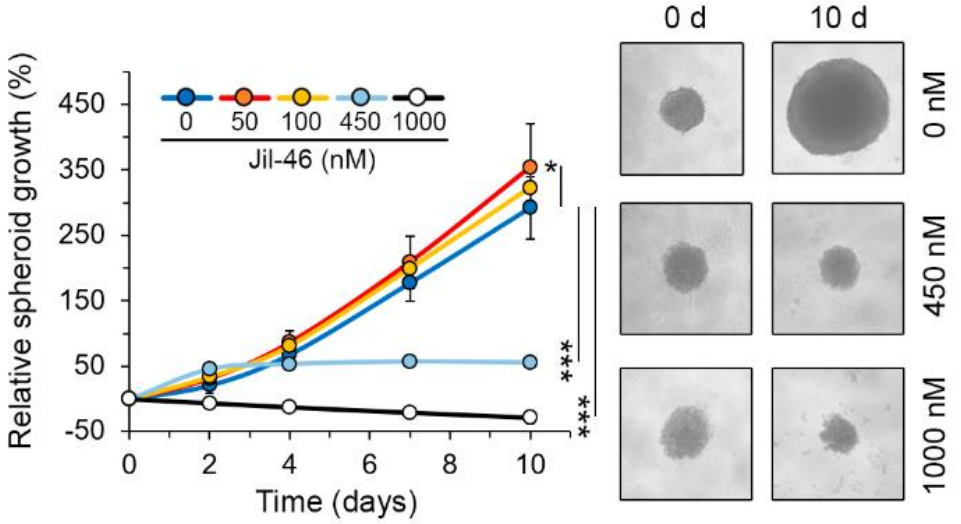
Jil-46 can bind to the active sites of TGF-β type I and type II receptors



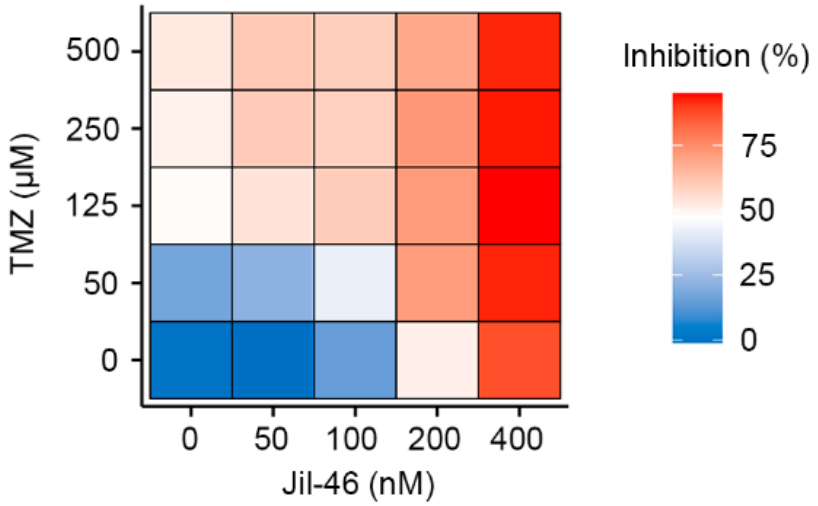


Jil-46 inhibits clonogenicity and spheroid growth of U87 cells

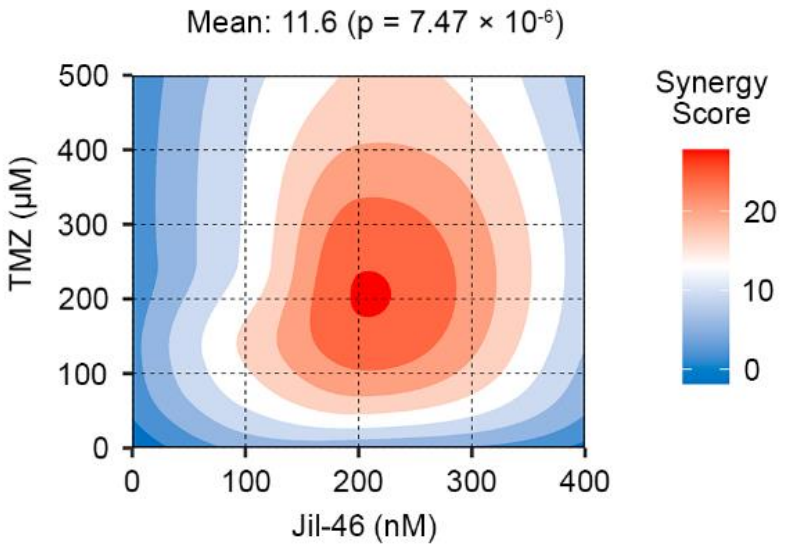




Jil-46 has a synergistic effect with temozolomide on U87 cell viability.

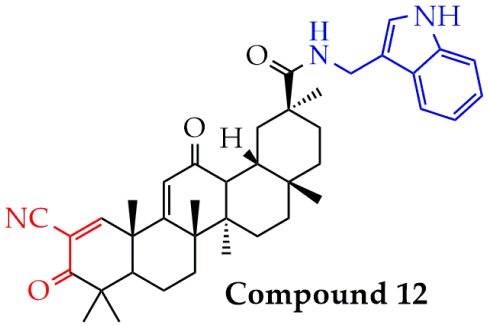


MTT assay data (72 h)



The synergy score was determined using SynergyFinder Plus

Cyanoenone-bearing triterpenoids are promising drug candidates for glioblastoma treatment



Compound 12

- Successfully permeable through BBB
- Toxic concentrations (glioblastoma):
  - ↑ ROS-dependent cell death
  - Probable direct interaction with LonP1
  - ↑ Mitochondrial apoptosis
- Non-toxic concentrations (glioblastoma):
  - ↓ Clonogenic activity
  - ↓ Cell motility
  - ↓ Vasculogenic mimicry
- ↓ Growth of U87 glioblastoma in vivo
  - ↓ Tumor volume and weight
  - ↓ Ki67-positive cells
- Probable protein targets
  - LonP1
  - EGFR
  - ERBB2
  - AKT1

Jil-46

- TGF-β1-stimulated GBM cells
  - ↑ Cell adhesion
  - ↓ Cell spreading
  - ↓ Cell motility
  - ↓ Expression of mesenchymal markers
  - ↑ Expression of glial marker OLIG2
- GBM cells
  - ↓ Clonogenic activity
  - ↓ Spheroid growth
  - ↑ Synergistic effect with TMZ
- Probable protein targets
  - TβRI
  - TβRII

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PhD student Okhina A. A.

RSF | Russian Science Foundation

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