Dual Inhibition of PDK1 and Aurora Kinase A: An Effective Strategy to Induce Differentiation and Apoptosis of Human Glioblastoma Multiforme Stem Cells

Simona Daniele, Simona Sestito, Deborah Pietrobono, Chiara Giacomelli, Grazia Chiellini, Danilo Di Maio, Luciana Marinelli, Ettore Novellino, Claudia Martini*, and Simona Rapposelli*

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Wipf Group Current Literature

Chaemin Lim

11/19/2016

Glioblastoma Multiforme (GBM)

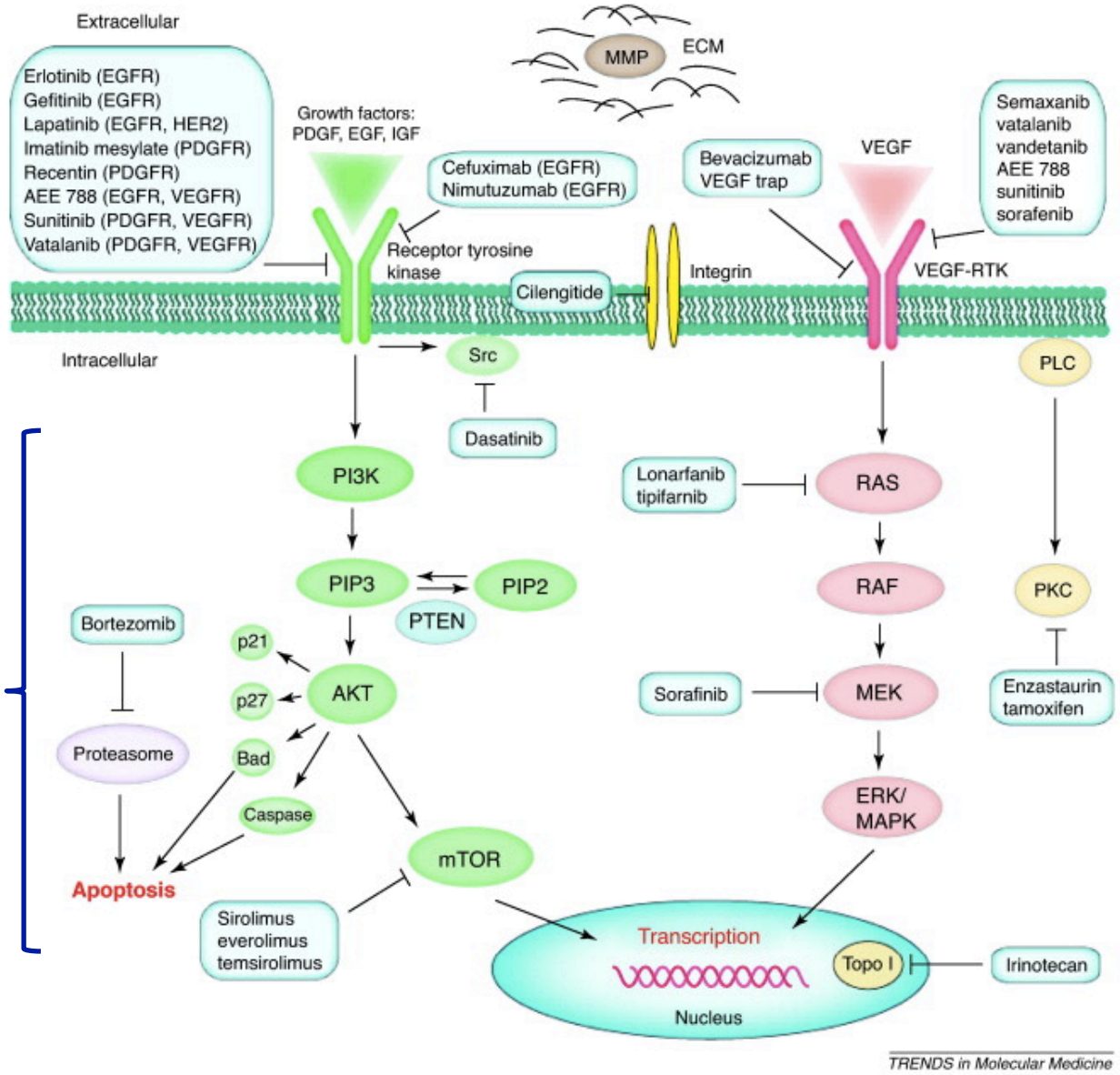
- A grad IV glioma; the most common and most aggressive malignant primary brain tumor
- Median survival rate of approximately 14 month, the 5 year survival rate for GBM is less than 5% in adult

- Current treatment relies on maximal surgical removal, along with radiotherapy and chemotherapy. But the rate of recurrence and therapeutic resistance is extremely high and it remains an incurable disease.
- The poor prognosis of GBM is mainly attributed to **drug resistance mechanisms** and to the existence of a **subpopulation of glioma stem cells (GSCs)**

- Aggressiveness and unresponsiveness of glioma have been correlated with the number of GSCs, and long-term TMZ treatment has been shown to favor the emergence of drug-resistant GBM cells, indicating that a **stem cell-oriented therapy is needed** to prevent GBM recurrence and to improve the outcome of treatments.

Current Molecular Targeted Therapies of GBM

- The incidence of GBM has been related to genetic and molecular alteration of different signaling pathways

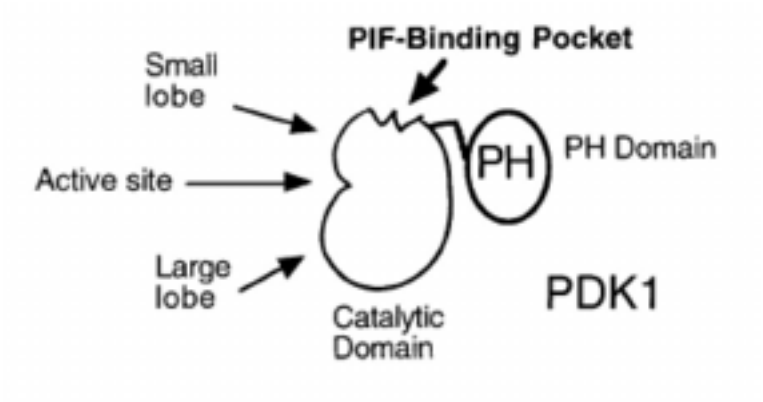


- The PI3K/Akt pathway: an important oncogenic pathway in the malignant phenotype of GBM
- Triggers a cascade of downstream signaling events that leads to tumor growth, survival, invasion into normal brain, and secretion of angiogenic factors.
- Greater activation and deregulation of PI3K/Akt components appears to increase cell proliferation and inhibit GSCs differentiation, thus contributing to resistance to chemotherapy.

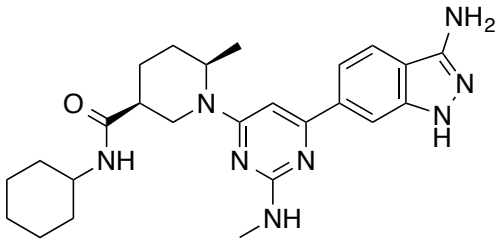
Trends in Molecular Medicine **2011**, *17*, 301-312.

PI3K/PDK1/Akt Signaling Pathway

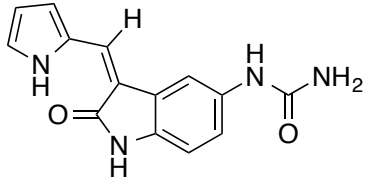
- The PI3K/PDK1/Akt signaling axis is centrally involved in controlling cell growth, proliferation, survival, tissue invasion and angiogenesis
- In particular, the 3-phosphoinositide-dependent kinase-1 (PDK1) inhibition has been suggested to block the oncogenic cellular processes



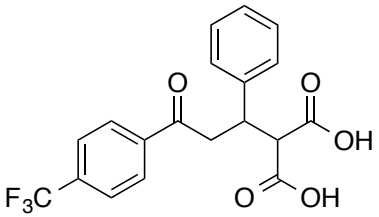
Domain structure of PDK1 indicating where the PIF-binding pocket is located on the small lobe of the kinase domain.
(Source: *The EMBO J.* **2001**, *20*, 4380-90)



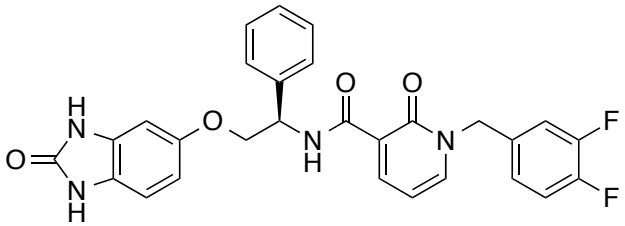
GSK2334470
ATP-binding site inhibitor
PDK1 IC_{50} = 2.5 nM



BX-517
ATP-binding site inhibitor
PDK1 IC_{50} = 6 nM



PS210
PIF pocket inhibitor
AC_{50} (Thr-308tide) = 2 μM

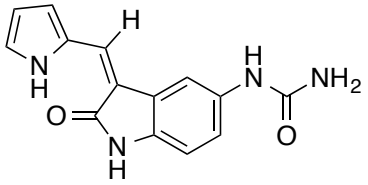


MP7
PDK1 inhibitor (DFG-out inhibitor)
IC_{50} (PDK1) = 6 nM

- However, specific target drugs, including those against PDK1, did not show a significant clinical efficacy

New OXID-pyridonyl hybrids as PDK1 Inhibitors

*Structures of two representative 2-oxindole-derivatives (OXIDs)



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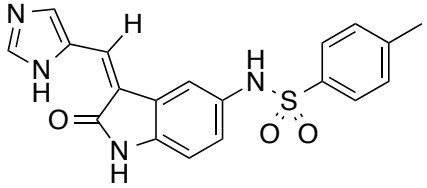
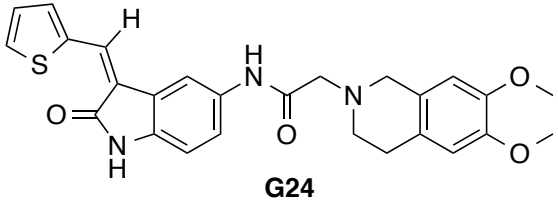
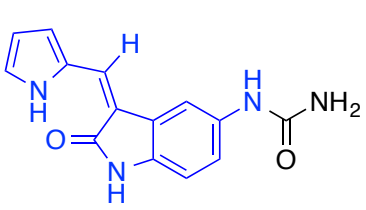


FC85

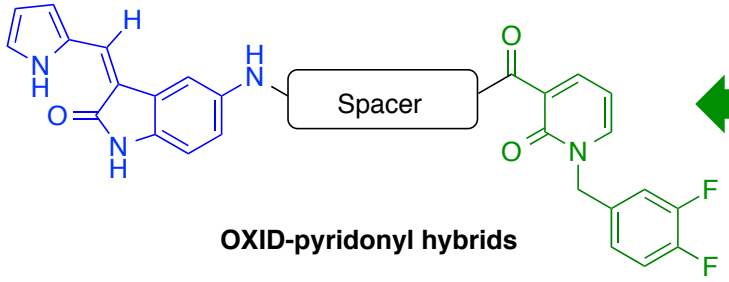


G24

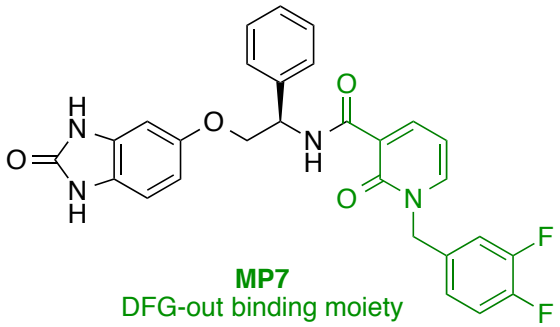
*Design strategy pursued to synthesise new OXID-pyridonyl hybrids



BX-517
ATP-binding site moiety



OXID-pyridonyl hybrids



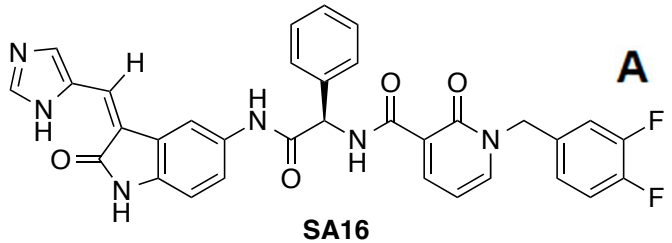
MP7
DFG-out binding moiety

- 2-oxindole part is well known to bind the hinge region of PDK1
- 2-oxo-pyridonyl fragment of MP7 interacts with the DFG-out binding site of PDK1
- Among these hydrids, SA16 inhibited both the PDK1 and AurA kinases at once

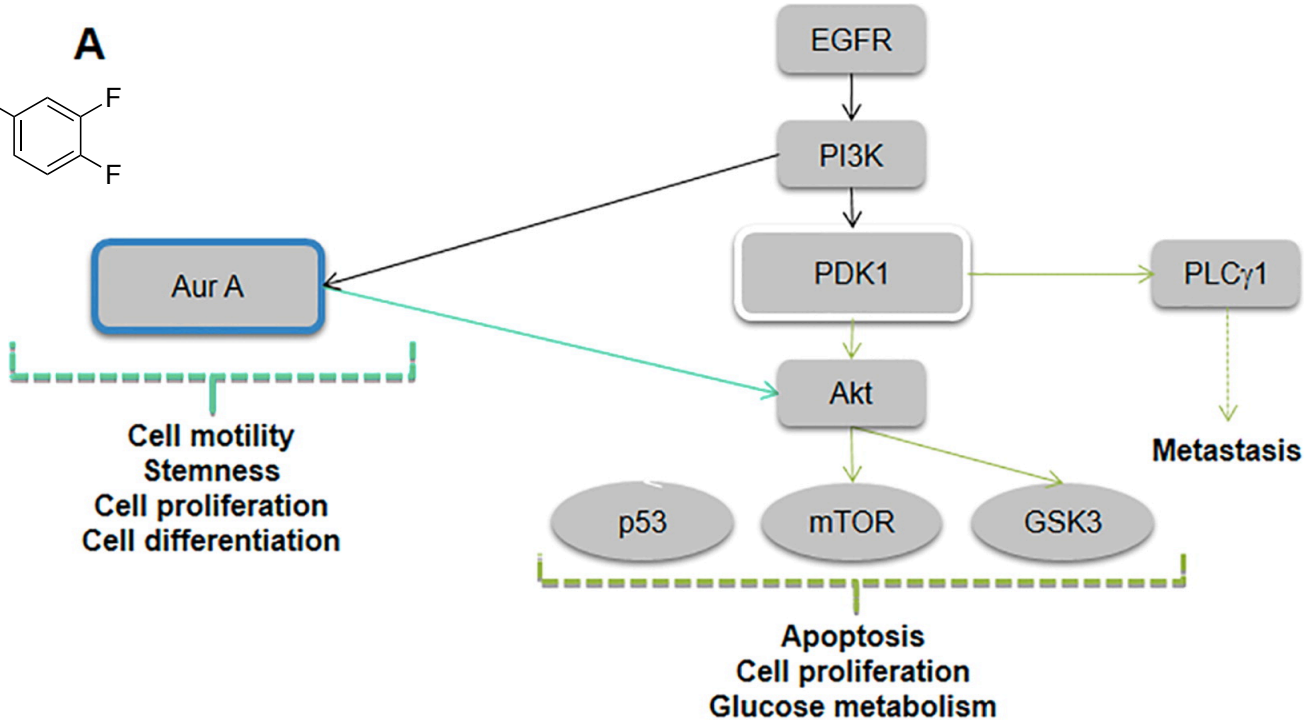
Eur J. Med. Chem. **2016**, 118, 47-63

Aurora Kinase A (AurA) - An Emerging Target

- Aurora Kinase A: A serine-threonine kinase that plays a pivotal role in cellular proliferation and differentiation of several tumors, including GBM
- Blocking the AurA pathway resulted in inhibition of GSC colony formation
- The simultaneous disruption of PDK1 and AurA could represent an innnovative strategy to overcome GBM resistance and recurrence
- A few PDK1 inhibitors also affect AurA activity: OS-03012 was found to affect multiple cellular targets, including AurA one, in neuroblastoma and other cancer cells



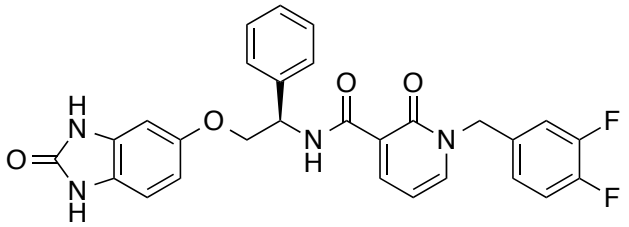
SA16
PDK1 inhibitor
IC_{50} (PDK1) = 416 nM
IC_{50} (AurA) = 35 nM



ACS Chem. Neurosci. **2016**, ASAP DOI: 10.1021/acschemneuro.6b00251

In this Study

A. The standard PDK1 inhibitor, MP7, and the AurA blocker Alisertib (MLN8237) were used as reference compounds to investigate the functional crosstalk between the two pathways in GBM



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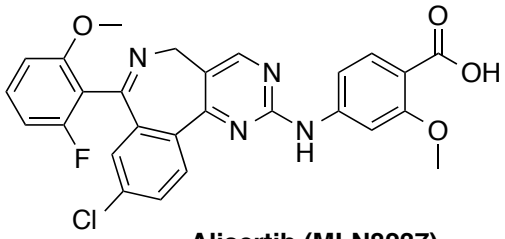
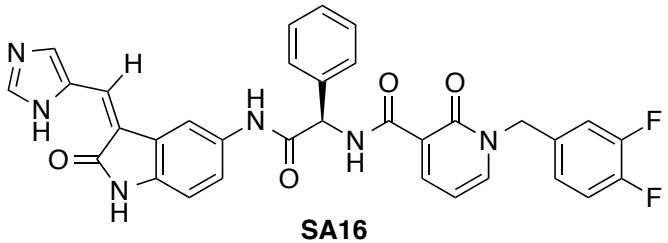


Alisertib (MLN8237)
AurA blocker
IC_{50} = 1 nM

B. SA16 (previously synthesized PDK1 inhibitors, namely OXID-pyridonyl hydrids) was identified as a new ligand able to inhibit both the PDK1 and the AurA pathways at once and thus useful in establishing the preclinical proof of mechanism for the simultaneous inhibition of these two pathways



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The combined inhibition of PDK1 and AurA affects GBM cell proliferation

- U87MG cells were chosen as representative GBM cell line - i) the lack of the tumor suppressor phosphatase and tensin homologue (PTEN), a negative regulator of the Akt pathway; ii) the expression of a functional AurA

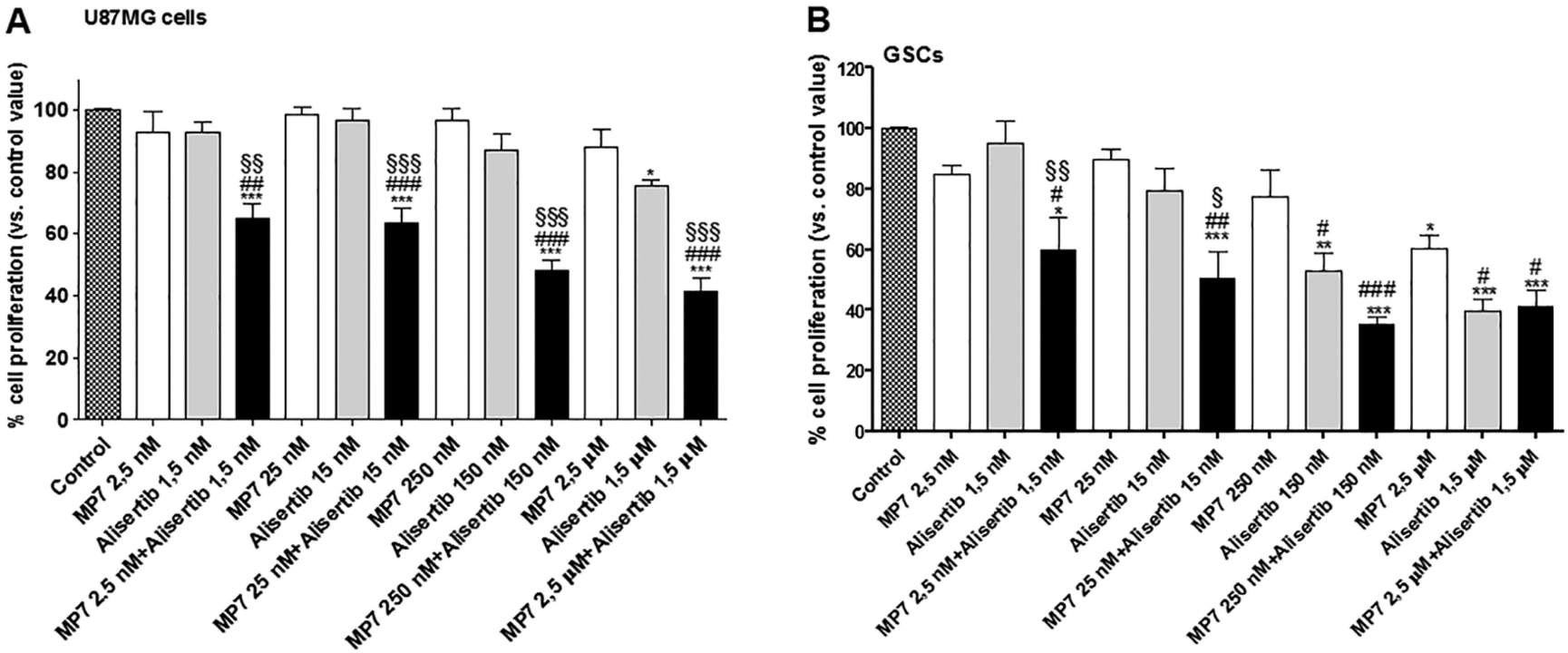


Figure 2. Effects of PDK1 and AurA inhibition on GBM and GSC proliferation. (A, B) U87MG cells (A) or GSCs (B) were incubated with the indicated concentrations of the PDK1 inhibitor MP7 or the AurA inhibitor Alisertib, alone or in combination, for 72 h or 7 days, respectively. At the end of treatment, cell proliferation was evaluated as described in Methods. The data are expressed as a percentage with respect to that of untreated cells (control), which was set to 100% (mean values ± SEM, N = 3). *p < 0.05, **p < 0.01, ***p < 0.001 vs control cells; #p < 0.05, ##p < 0.01, ###p < 0.001 vs cells treated with MP7 alone; §p < 0.05, §§p < 0.01, §§§p < 0.001 vs cells treated with Alisertib alone.

The combined inhibition of PDK1 and AurA affects GSC proliferation and induces their differentiation

- Both PDK1 and AurA have been demonstrated to play an important role in GSC survival/ differentiation.
- The effect of MP7, Alisertib and their combination on GSC morphology were evaluated.
- The two compounds, then administered individually, led to a reduction in the area occupied by the neurospheres

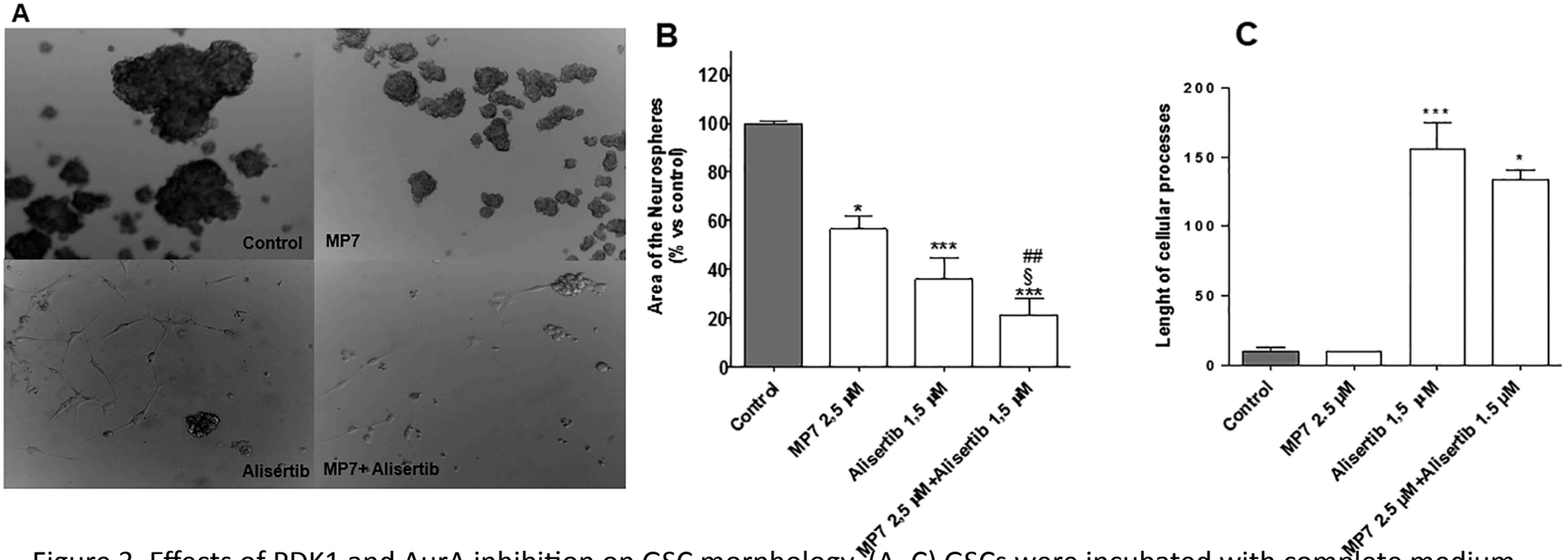
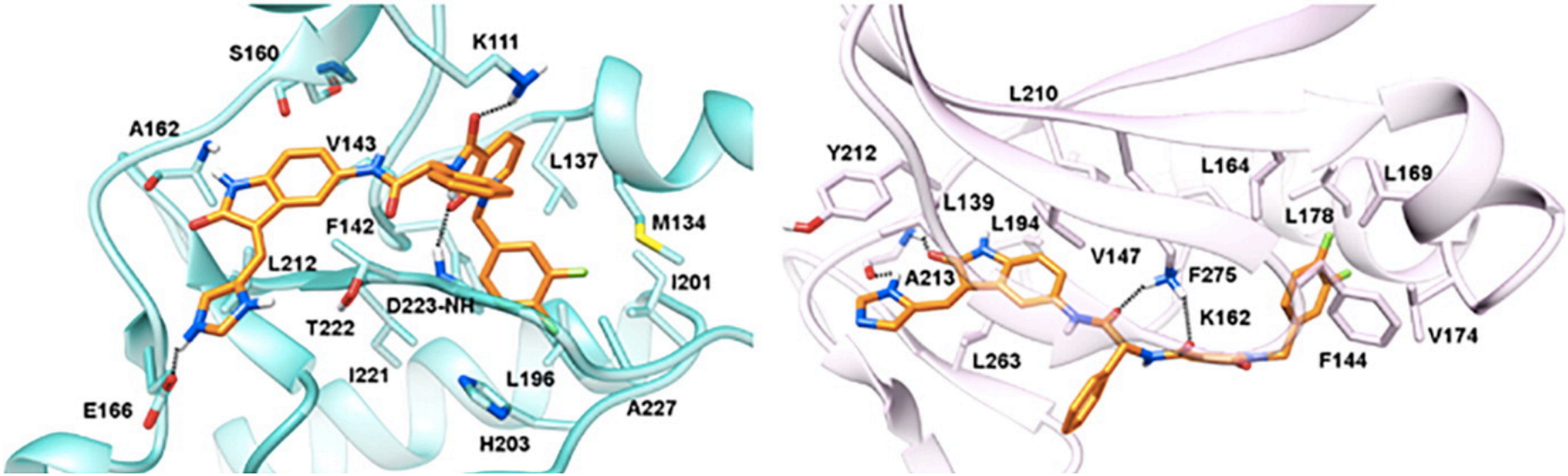


Figure 3. Effects of PDK1 and AurA inhibition on GSC morphology. (A–C) GSCs were incubated with complete medium containing DMSO (control), 2.5 µM MP7, and 1.5 µM Alisertib, alone or in combination, for 7 days. (A) Representative cell micrographs after 7 days of treatment are shown. The area of the culture plates occupied by the spheres (B) and the length of cellular processes (C) were scored after 7 days of treatment (mean values ± SEM, N = 3). *$p < 0.05$, ***$p < 0.001$ vs control, ##$p < 0.01$ vs MP7 alone, §$p < 0.05$ vs Alisertib alone.

Molecular Modeling - Binding mode of SA16

A Interactions with 3,4-difluorobenzene ring, 2-pyridone ring, amidic carbonyl, etc

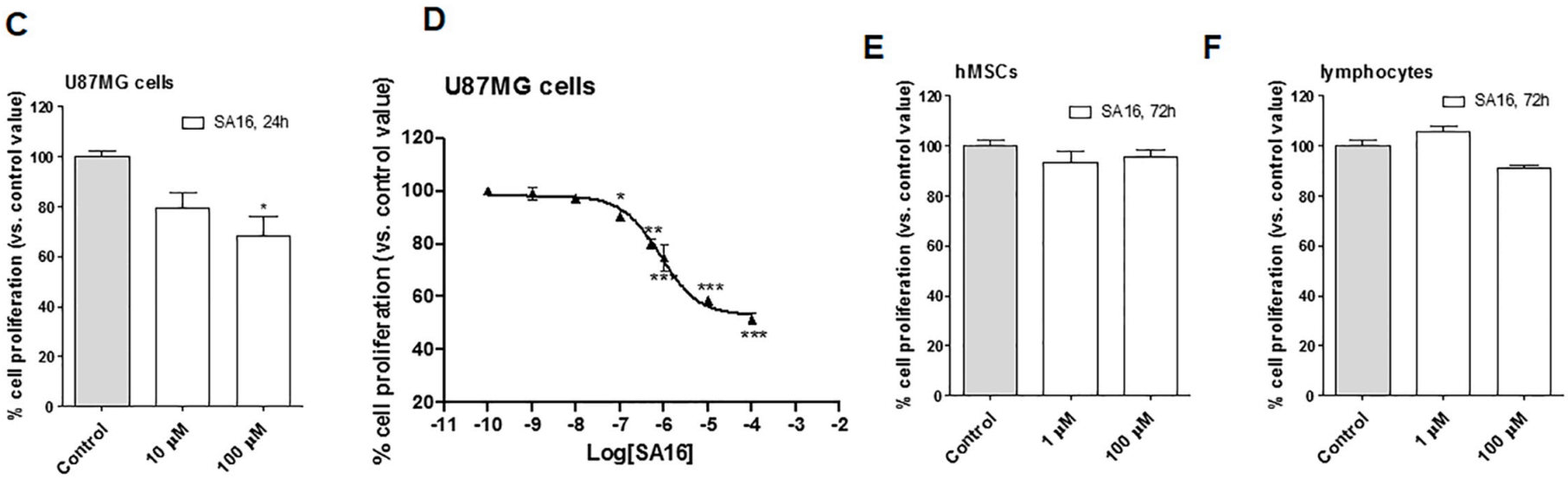
B AurA conformation that binds to SA16 is not known
DFG-out pocket of AurA is narrower



(A, B) Binding mode of SA16 as found by the docking calculations within its (A) PDK1 and (B) AurA biding sites. Both SA16 (colored in orange) and the protein residues involved into binding are depicted as sticks. The structures of PDK1 and AurA are depicted as cartoons, colored in cyan and pink, respectively.

Effects of SA16 on Cancer Cell vs Normal Cells

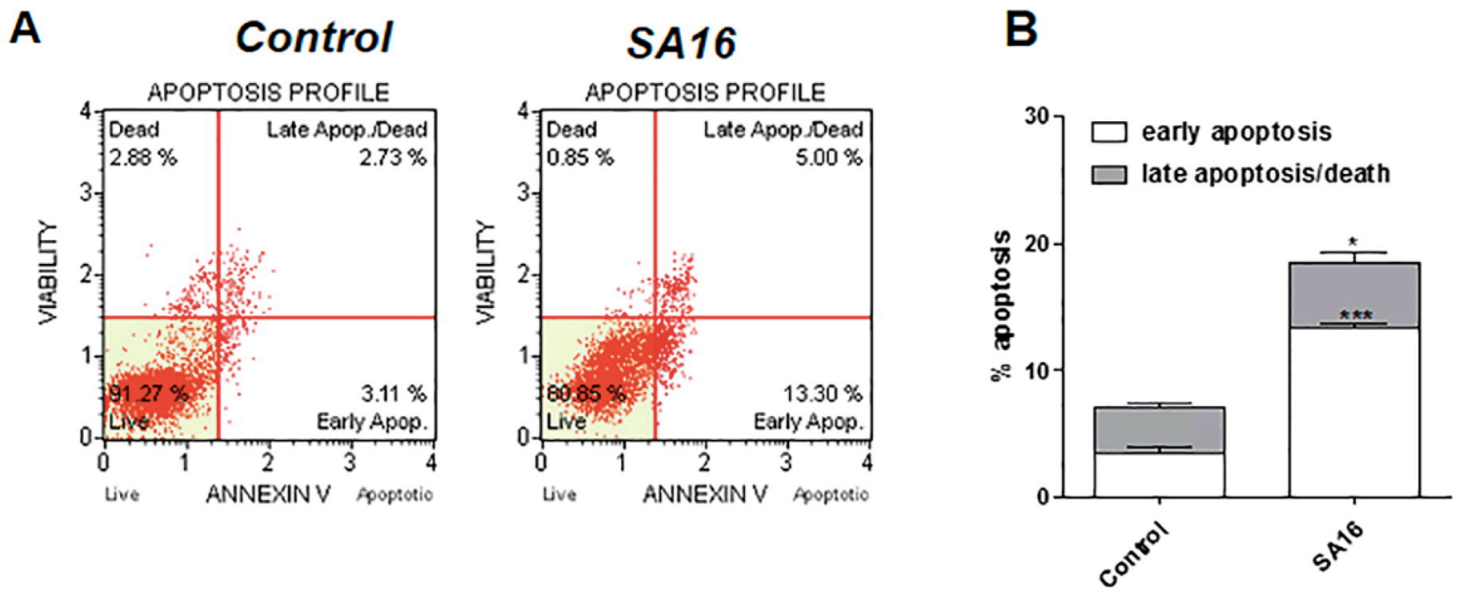
- The dual-target compound blocks GBM proliferation, but lacks toxicity in normal cells



(C, D) U87MG cells were treated in complete medium with the indicated concentrations of SA16 for 24 h (C) or 72 h (D), and cell proliferation was evaluated as described in the Methods. (E, F) Human MSCs (E) or lymphocytes (F) were treated with DMSO or the indicated SA16 concentrations. Following treatment, cell proliferation was evaluated as described in the Methods. The data are expressed as a percentage with respect to that of untreated cells (control), which was set to 100% (mean values ± SEM, N = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control.

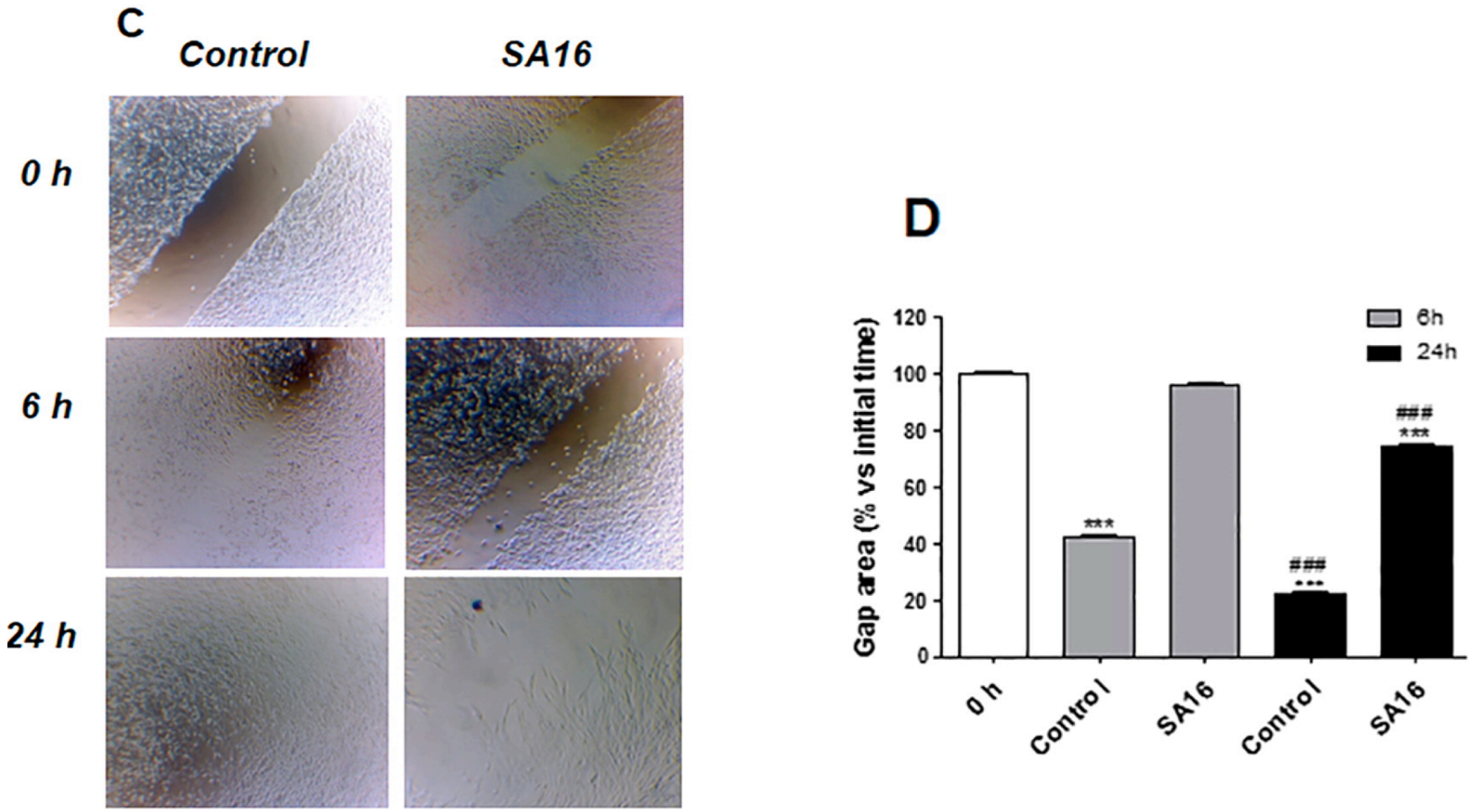
SA16 Induces GBM Cell Apoptosis

- The effect on GBM proliferation/viability was accompanied by cellular apoptosis



(A, B) U87MG cells were incubated with DMSO (control) or 10 µM SA16 for 72 h. At the end of the treatment period, the cells were collected, and the level of phosphatidylserine externalisation was evaluated using the Annexin V-staining protocol. (B) Data are expressed as the percentage of apoptotic cells versus the total number of cells (mean values ± SEM, N = 3). The data for the early stage apoptotic cells are shown in white, while the data for the late-stage apoptotic/necrotic cells are shown in gray. *p < 0.05, ***p < 0.001 vs control.

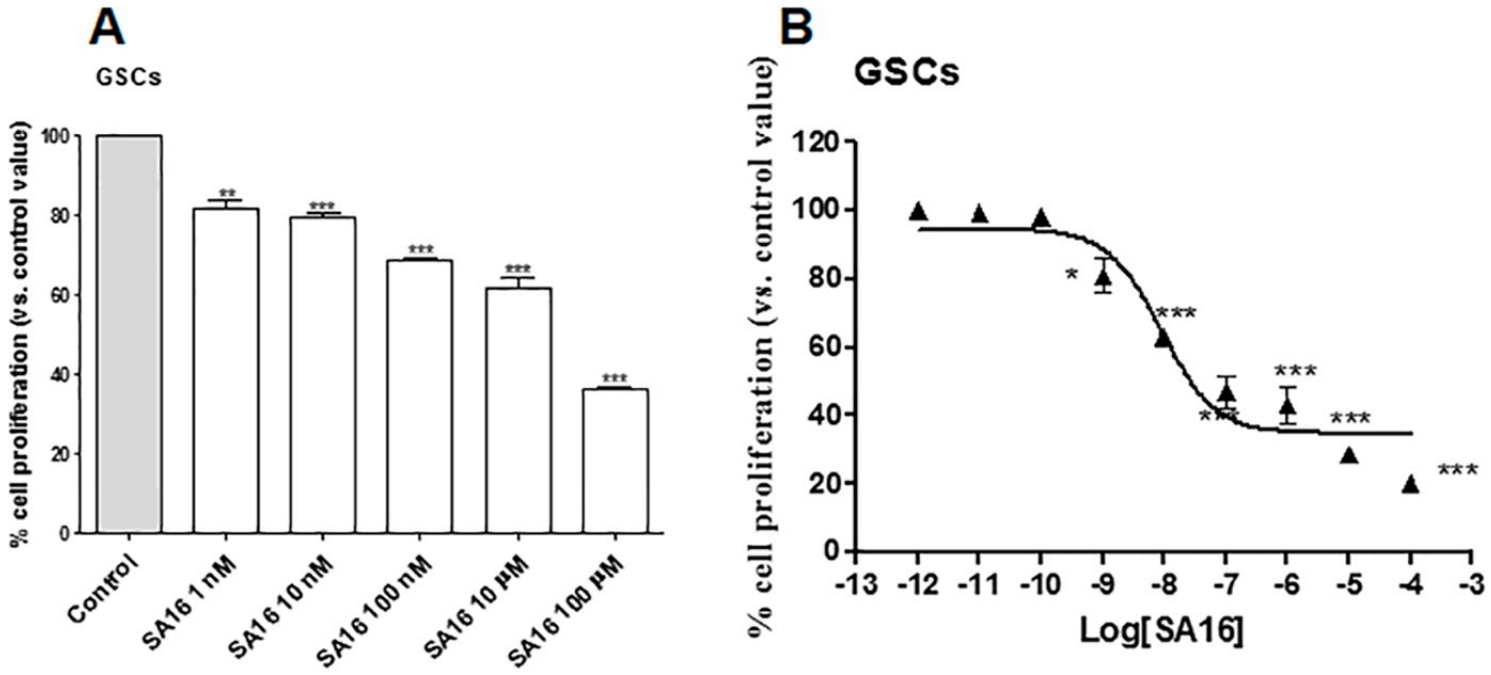
SA16 Inhibits Cell Migration: Wound Healing Migration Assay



(C, D) U87MG cells were treated for 72 h with DMSO (control), or 10 µM SA16. Following treatment, the cell monolayer was scratched (time zero), and cells were grown in fresh medium. Representative micrographs were taken after 6 and 24 h from the scratch (C). The gap area (D) was measured after 6 and 24 h from the scratch (mean values ± SEM, N = 3). ***p < 0.001 vs control; ###p < 0.001 vs respective gap area at 6 h from the scratch.

SA16 Inhibits GSC Formation and Viability

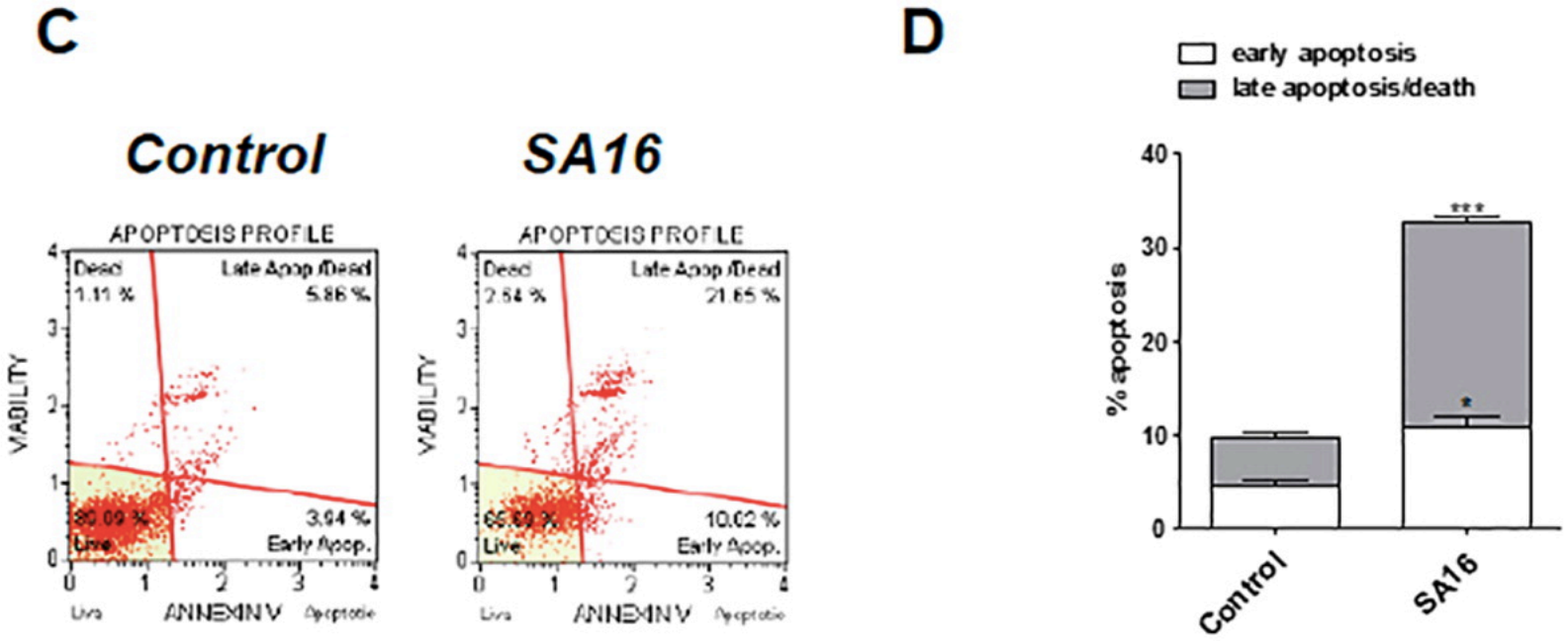
- SA16 induced a concentration-dependent inhibition of GSC proliferation, starting after 4 days of cell incubation
- Following a 7 day treatment, SA16 yielded an IC_{50} value of 8.33 ± 0.78 nM and a maximal percentage of 80.0 ± 2.0%



(A, B) U87MG-derived GSCs were incubated with different concentrations of SA16 for 4 days (A) or 7 days (B).

SA16 Inhibits GSC Formation and Viability

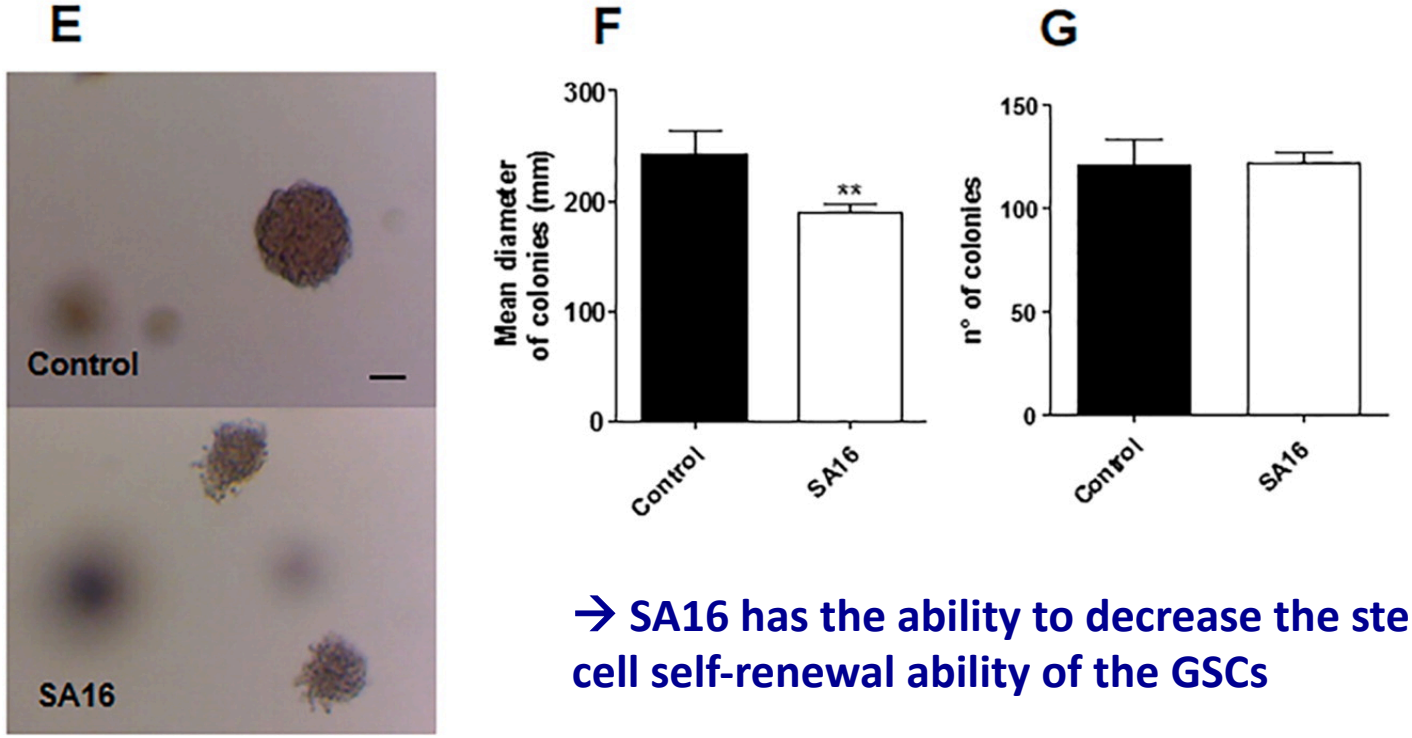
- The SA16-mediated reduction of GSC proliferation was associated with apoptosis/death, as demonstrated by the significant induction of phosphatidylserine externalization in the presence of 7-AAD binding to DNA
- **The dual target ligand is able to arrest GSC proliferation and induce their apoptosis**



(C, D) GSCs were treated as in (B), and the apoptosis degree was evaluated using the Annexin V protocol. (C) Data were expressed as the percentage of apoptotic cells (early apoptotic is represented in white, late-apoptotic/necrotic in gray) relative to the total number of cells (mean values ± SEM, N = 3).

SA16 Decreases the stem cell self-renewal ability of the GSCs

- GSC cells were dissected and a soft-agar assay was performed
- The effect of a subtoxic dose of SA16 (1 nM) was evaluated after 21 days of treatment.
- SA16 did not affect the total sphere number (Figure E and G)
- The diameter of the newly formed spheres was significantly reduced in the presence of SA17 (Figure E and F)

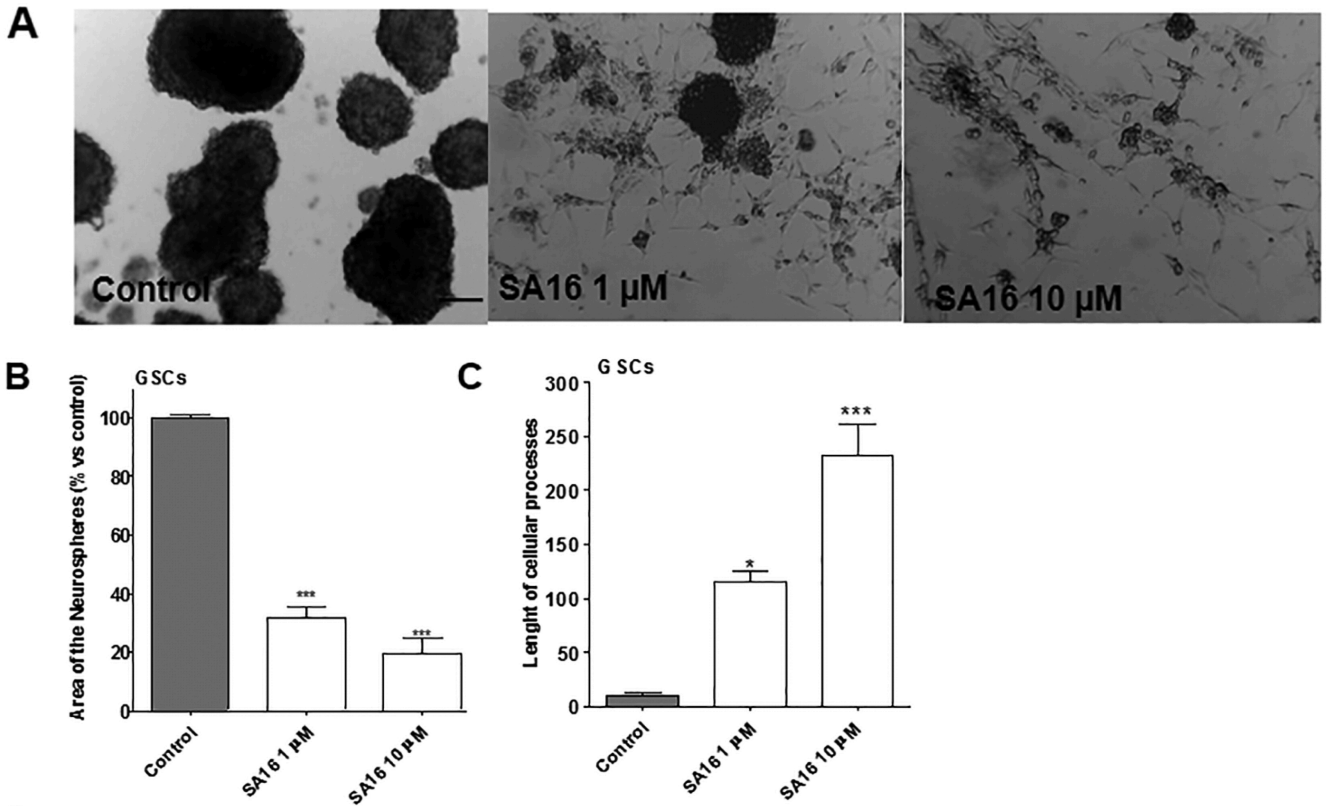


→ SA16 has the ability to decrease the stem cell self-renewal ability of the GSCs

(E) Representative pictures of the cells after 21 days of incubation. Mean diameter (F) and number of the newly formed spheres (G) were scored. *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

SA16 Affects GSC Morphology

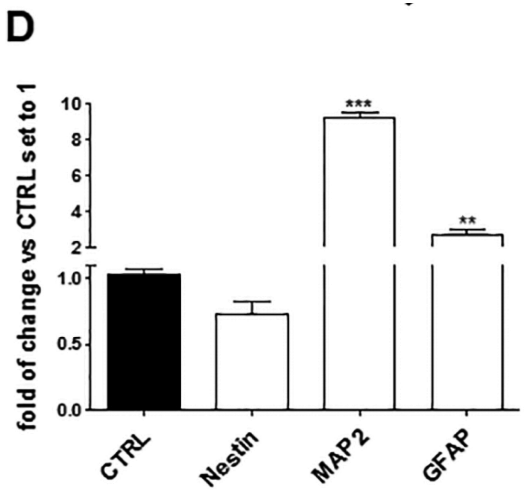
- The effects of SA16 on GSC morphology were evaluated by quantifying the area occupied by the cells in the culture plates. GSCs were incubated for 7 days with complete medium containing DMSO (control) or the indicated concentrations of SA16.



(A) Representative cell micrographs after 7 days of treatment. The area occupied by the cells in culture plates

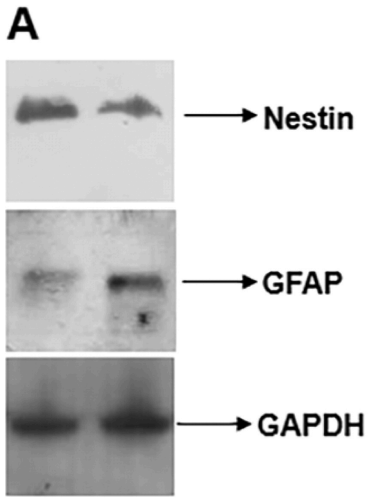
(B) and the outgrowth of cellular processes (C) were scored after 7 days of treatment (mean values ± SEM, N = 3). The data are expressed as the fold change vs the levels of the control (mean values ± SEM, N = 3). *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

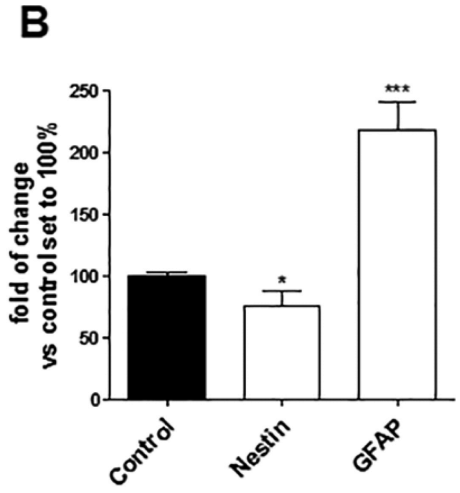
SA16 Reduces GBM Stemness Potential

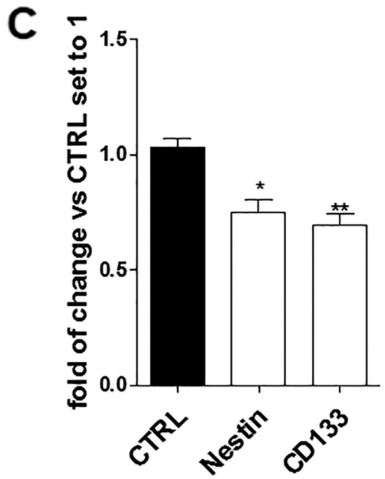


(D) Total RNA was extracted, and the relative mRNA quantification of the stem cell marker Nestin, the neuronal marker MAP2, and the astrocyte marker GFAP was performed by RT-PCR.

- Real time PCR experiments showed that the compound induced a significant transcription of the glial marker GFAP and of neuronal marker MAP2.
- SA16 induced stem cell differentiation toward a neuronal and glial phenotype.



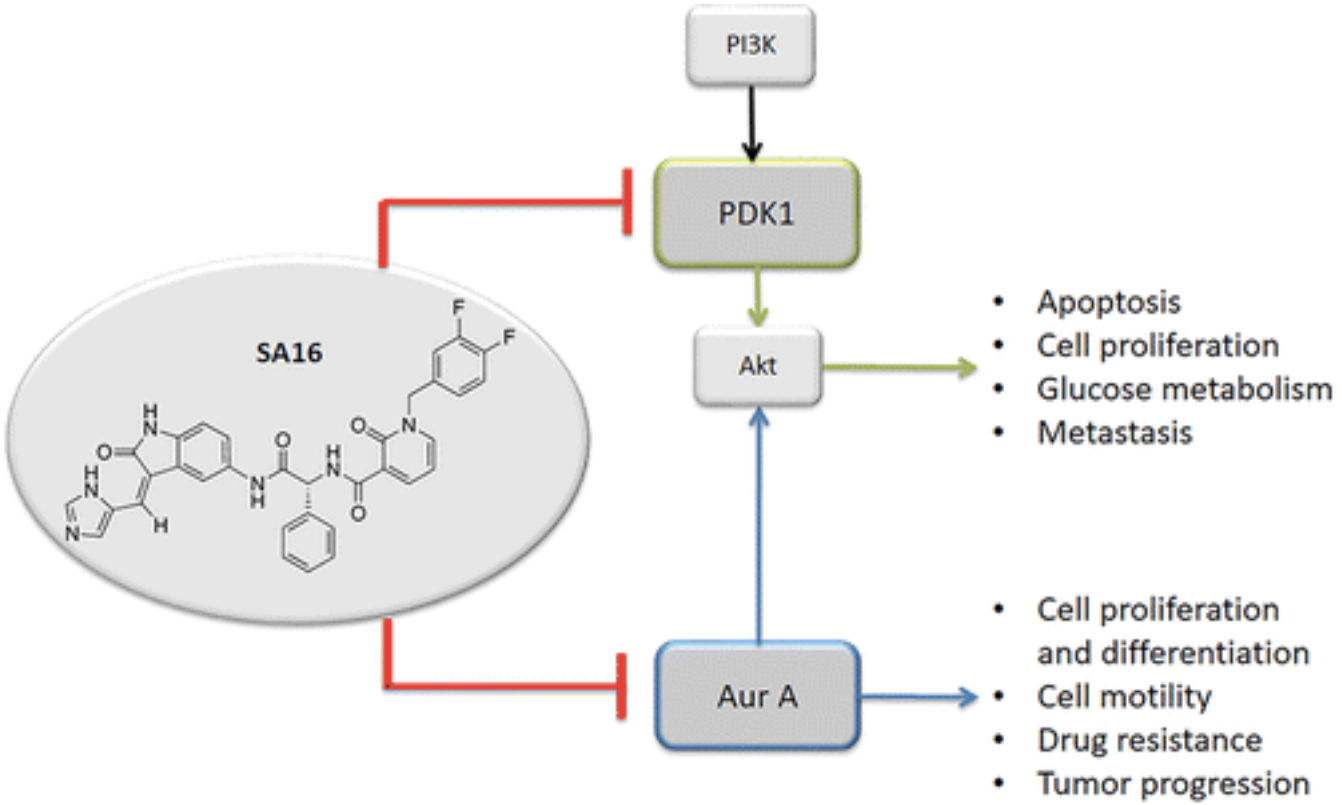




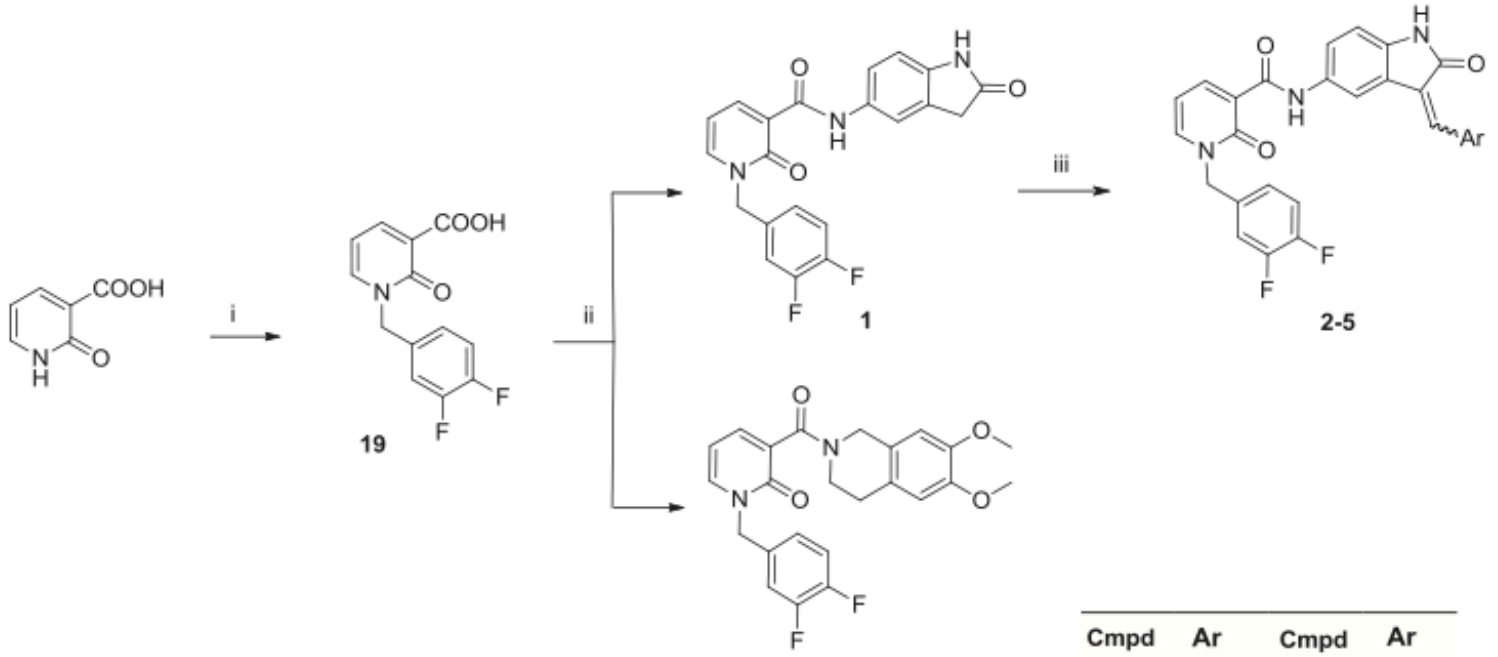
(C) Stem cell markers were analyzed in U87MG cells treated with SA16 for 72 h, to see if adherent GBM cells could lose their stemness potential after drug treatment

(A, B) GSCs were treated with DMSO (control) or 10 µM SA16 for 7 days; the protein levels of the stemness marker Nestin and of the glial marker GFAP were assessed by Western blotting, using GAPDH as the loading control. (C) U87MG were incubated with SA16 for 72 h. (C) Total RNA was extracted, and the relative mRNA quantification of the stem cell markers Nestin and CD133 was performed by RT-PCR. Data are expressed as the fold change vs the levels of the control (mean values ± SEM, N = 3). *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

Summary

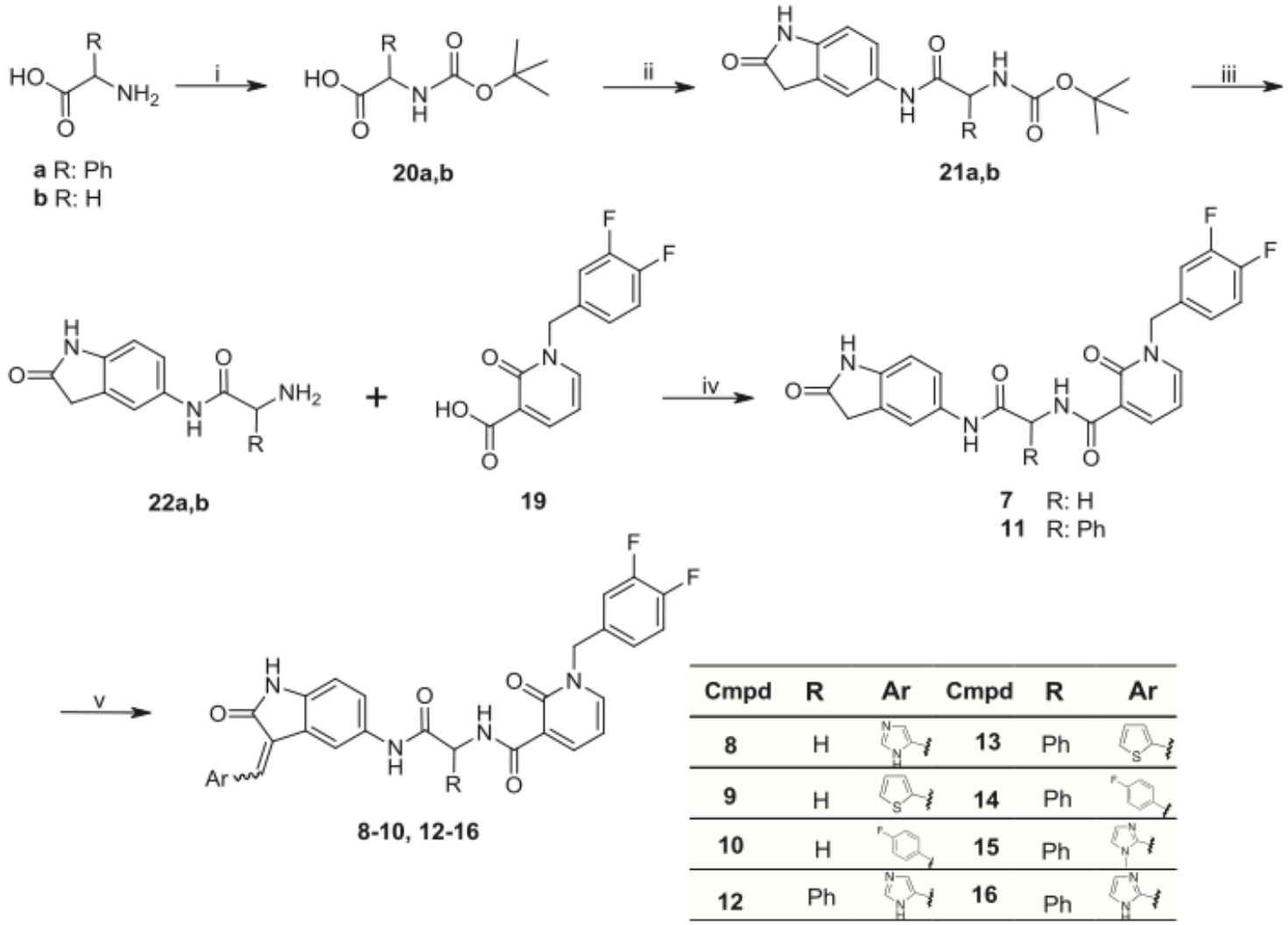


- The first study reporting a combinatorial treatment strategy based on the simultaneous inhibition of PDK1 and AurA
- Identified an innovative PDK1/AurA dual-target molecule which could represent an attractive **lead scaffold for the design and synthesis of new multi-taget treatments for GBM and GSCs**
- Brain penetration? / in vivo study / PK parameters



Cmpd	Ar	Cmpd	Ar
2		4	
3		5	

Scheme 1. [a]Reagents and Conditions: *i)* 3,4-difluoro-benzylbromide, NaH 60%, dry DMF, 50 °C, 16 h; *ii)* THI or 2-oxo-5-amine-indole, TBTU, DIPEA, dry DMF, 0 °C → r.t., 12 h; *iii)* Appropriate carbaldehyde, absolute EtOH, piperidine, 110 °C, 4 h.



Cmpd	R	Ar	Cmpd	R	Ar
8	H		13	Ph	
9	H		14	Ph	
10	H		15	Ph	
12	Ph		16	Ph	

Scheme 2. [a]Reagents and Conditions: *i)* $(Boc)_2O$, NaOH 1 M/iPrOH 4:3, r.t., 2 h; *ii)* 5-amino-2-oxo-indole, TBTU, DIPEA, dry DMF, r.t., 16 h; *iii)* TFA, DCM, −10 °C/−20 °C, 3 h; *iv)* TBTU, DIPEA, dry DMF, r.t., 16 h; *v)* Appropriate carbaldehyde, iPrOH, DMF, piperidine, 110 °C, 4 h.