NOVEL BENZENESULFONATE SCAFFOLDS WITH A HIGH ANTICANCER ACTIVITY AND G2/M CELL CYCLE ARREST IN GLIOBLASTOMA MULTIFORME

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour in adults, encompassing 16% of all primary brain neoplasms. GBM belongs to the most clinically aggressive cancers, which are characterized by poor prognosis. The median survival of patients with GBM is 12–15 months with a 5-year survival rate that remains at less than 5%, despite the use of intensive treatment modalities. Current therapy consists of surgical resection, radiotherapy together with concomitant chemotherapy. Innovative treatment strategies that have been implemented have contributed to extending the survival time, which is still far from satisfactory. For this reason, exploring and designing a novel and effective anticancer agents or protein inhibitors is still leading challenge in drug development.

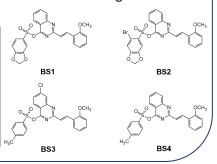
Sulfonates, unlike their derivatives, sulphonamides, have rarely been investigated for their anticancer activity. Unlike the well-known sulphonamides, esters are mainly used as convenient intermediates in synthesis. Here, we present the first in-depth investigation of quinazoline sulfonates. Based on their structural similarity, these compounds resemble tyrosine kinase inhibitors and the p53 reactivator CP-31398, but their mechanism of action is quite different.

ANTIPROLIFERATIVE ACTIVITY TOWARDS GLIOBLASTOMA CELLS

The ability of the newly synthesized compounds to inhibit the proliferation of the human glioblastoma cells was verified on a panel of four cell lines with different genetic and protein profiles. We focused on U87 cells (with p53 wild type and PTEN mutation), U-251 cells (with p53 and PTEN mutations), T98G cells (with p53 and PTEN mutations), and DKMG/EGFRvIII cells (with EGFR mutation). In general, the analyzed tosylates expressed a high submicromolar activity against GBM cells. Among them, the **BS3** derivative appeared to be the most active. It is worth noting that three of the tested sulfonates of quinazolines were selective against human normal glial cells - astrocytes.

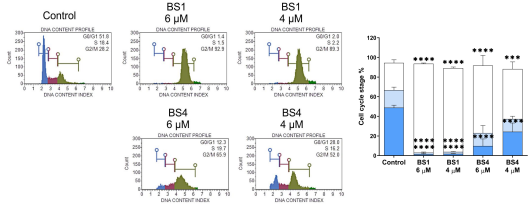
Cell line / Comp.	Antiproliferative activity – IC_{50} [μM]				
	U-87	U-251	T98G	DKMG/EGFR vIII	NHA*
BS1	1.439 ± 0.122	1.897 ± 0.649	1.647 ± 0.304	0.499 ± 0.037	>25
BS2	0.921 ± 0.161	2.303 ± 0.234	NT	0.343 ± 0.020	>25
BS3	0.430 ± 0.100	1.757 ± 0.388	NT	0.432 ± 0.030	3.463 ± 0.463
BS4	1.310 ± 0.224	1.907 ± 0.214	3.065 ± 0.643	0.422 ± 0.084	>25

*NHA – normal human astrocytes

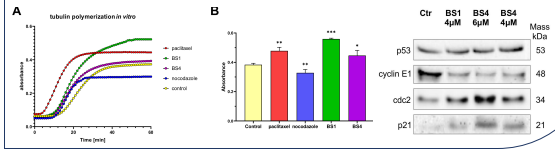


CELL CYCLE ARREST IN U-251 CELLS

We selected two candidates for a more in-depth analysis of the molecular mechanism of action of the sulfonic styrylquinazoline derivatives: **BS1** and **BS4**. As presented below, the percentage of the cells that were arrested in the G2/M phase increased in a concentration-dependent manner for both sulfonic derivatives. For example, at 6 μM, **BS1** caused a remarkable accumulation of cells in the G2/M phase to 90.68% (from 27.78% in the untreated cells), which was accompanied by a significant decrease in the number of cells in the G0/G1 and S phases to 1.52%. Notably, the observed strong cell cycle arrest effect in the G2/M phase by the **BS1** derivative was greater than that of the antimitotic drug paclitaxel.

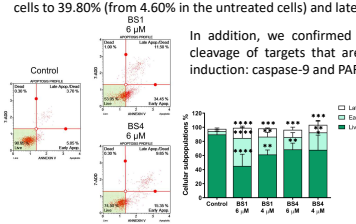


Moreover, the cell cycle arrest may be possibly, if not exclusively, associated with the interaction with microtubules. Our studies exhibited that tested tosylates caused a significant enhancement of tubulin polymerization. Interestingly, the observed effect was much stronger than that of the well-known enhancer of this process, paclitaxel. In addition, we indicated that the derivatives tested modulated the expression of cell cycle proteins: p53, cyclin E1, cdc2, p21.



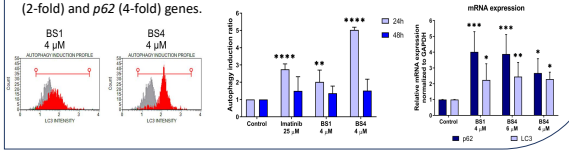
AUTOPHAGY AND APOPTOSIS INDUCTION IN U-251 CELLS

Apoptosis and autophagy were confirmed as the cell death modes that corresponded with the inhibition of metabolic activity and the activation of the p53-dependent and p53-independent pathways. Both tested sulfonates of quinazolines apoptosis induction to a similar extend. Namely, the highest apoptotic effect was observed when cells were exposed to **BS1** at a 6 μM concentration, in which there was a significant increase the percentage of early apoptotic cells to 39.80% (from 4.60% in the untreated cells) and late apoptotic cells to 11.40%.



In addition, we confirmed an increase in BID expression and cleavage of targets that are directly associated with apoptosis induction: caspase-9 and PARP.

As presented below, patterns of autophagy induction were visible in the U-251 cells. In particular, we observed a strong effect on autophagosome formation and autophagy induction after a 24 h treatment. The **BS4**, for which the calculated autophagy ratio was 5.03, had a higher effect. Additionally, autophagy was confirmed by examining the expression of *LC3* and *p62* genes. Namely, both tosylates caused a strong increase in the expression levels of *LC3* (2-fold) and *p62* (4-fold) genes.



CONCLUSION

The tested tosylates are characterized by high anticancer activity and strong cell cycle arrest at the G2/M phase in GBM cells. Moreover, the in-depth analysis revealed the multitargeted mechanism of action for these compounds Therefore, it can be concluded that the sulfonates of quinazolines can be regarded as promising scaffolds for developing anticancer agents. Additionally, this can be hypothesized that potent G2/M inhibitors may be effectively used for overcoming drug resistance or adverse effects, as is known for paclitaxel.

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