In Vitro Inhibition of Glioblastoma Cell Growth by N-substituted Imidooxy Derivatives

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BACKGROUND

- Glioblastoma (GBM) is the most common CNS tumor with a high mortality rate [1]
- Patients presenting with glioblastomas have an average median survival of 14 to 15 months [2]
- Despite unique multimodal treatment approaches, GBM tumors have developed resistance to conventional treatment modalities
- This study evaluates the in vitro effects of *N*-substituted imidooxy compounds on the growth of GBM cells and identifies the signaling pathways affected using this treatment

Cell Characteristics

- Heterogeneous tumors [3]
- Decreased apoptosis
- Increased cell proliferation
- The P13K/AKT pathway is a signaling pathway through which GBM cells proliferate [4]

Current Therapies

- Surgical debulking of the tumor, radiotherapy, and chemotherapy with temozolomide [5]
- The combination treatment modalities have not significantly prolonged the survival rate in glioblastoma patients

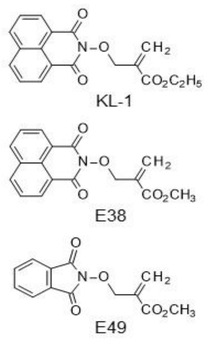

Figure 1: Structures of imidooxy compounds

METHODS

Cell Growth and Viability

- U251 and A172 GBM cells were treated with the imidooxy compounds for 48h to determine their effects on viability using the crystal violet assay. The absorbance was measured at 590 nm using a spectrophotometer.
- GBM cells were treated with the imidooxy compounds for 48h and 72h to determine their cytotoxic effects using the LDH cytotoxicity assay kit. The absorbance was measured at 490 nm using a spectrophotometer.
- A colony formation assay was done to determine the effects of the imidooxy compounds on GBM cell growth.
- An MTT assay was performed to assess the viability of GBM cells in the absence or presence of the imidooxy derivatives. The absorbance was read using a spectrophotometer at 560 nm.

Survival and Apoptotic Signaling Targets

- The expressions of survival proteins such as Akt and apoptotic proteins such as PARP, caspase, and Bcl-xL were analyzed via western blotting. Protein bands were visualized using the Azure biosystems 300Q.
- Flow cytometry was used to analyze the percentage of cells in the different apoptotic stages.

Data Analysis

- GraphPad Prism 9 was used for statistical analyses, and data represented as means ± S.E.M.
- One-way ANOVA was used.
- *p<0.05 indicates statistical significance.

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RESULTS

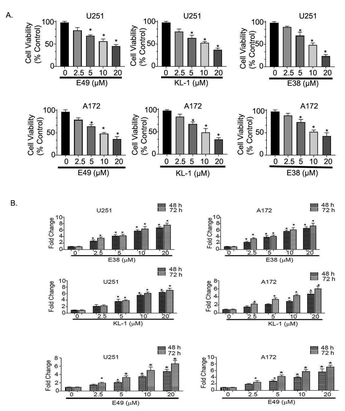

Figure 2: (A): Cell viability using crystal violet assay. (B): LDH assay

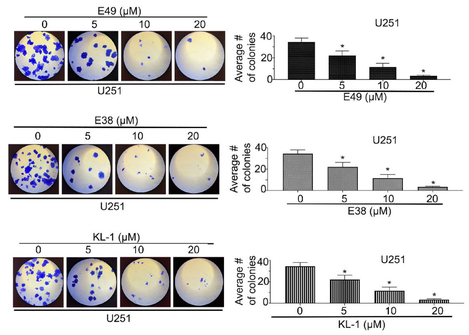

Figure 2: (C): Average number of colony formation in U251 cells after treatment with imidooxy compounds

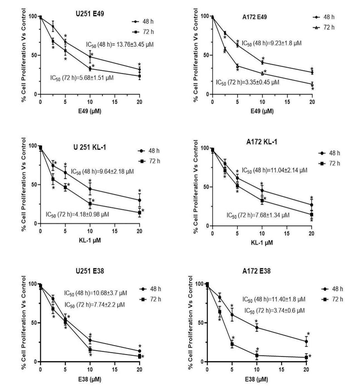

Figure 3: Cell proliferation as determined by MTT assay

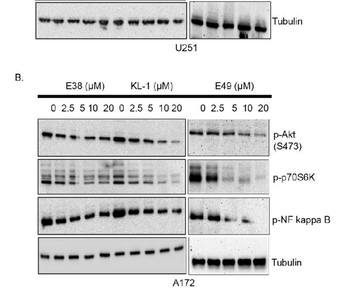

Figure 4: Effects of imidooxy compounds on the survival signaling targets. (A) U251 and (B) A172 GBM cell lines

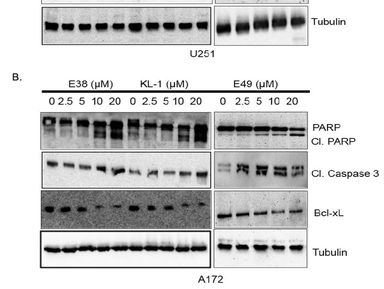

Figure 5: Effects of imidooxy compounds on the apoptotic cellular signaling targets. (A) U251 and (B) A172 GBM cell lines

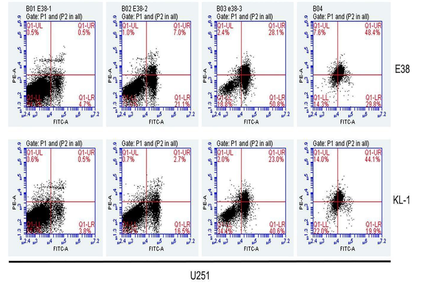

Figure 5: (C): The apoptosis of U251 and A172 examined using FITC-Annexin V and PI staining

DISCUSSION AND CONCLUSIONS

- This study found that the imidooxy derivatives exert an anticancer effect on glioblastoma cells by inhibiting cell viability, proliferation, colony formation, and induction of apoptosis
- The imidooxy derivatives showed an inhibitory effect on the proliferation of U251 and A172 glioblastoma cells by suppressing the p-Akt/p70S6K/p-NF kappa B signaling pathway in a concentration-dependent manner
- In vivo testing of the imidooxy derivatives is needed to validate their potential for decreasing tumor size and volume in NON-SCID mice.

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