

ANTIPROLIFERATIVE EFFECTS OF PYRAZOLO-PYRIMIDINE -TYPE INHIBITORS OF SRC FAMILY TYROSINE KINASES DERIVATES ON HUMAN NEUROBLASTOMA CELLS

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Neuroblastoma (NB) is the most common solid tumour in children. Despite many advances in cancer therapies, NB has remained a tumour that usually undergoes rapid progression with a fatal outcome. Numerous studies have identify the protein tyrosine kinases (TK) as targets for cancer therapy, since enhancement of TK activity has been correlated with proliferative diseases. Thus, several synthetic TK inhibitors have been developed and tested as anticancer drugs. These include the some pyrazolo-pyrimidine derivatives, able to selectively inhibit Src-family tyrosine kinases (Src-TK), a group of protein that has been involved in cancer development and invasivity. In this study we evaluated the effects of three novel pyrazolo-pyrimidine derivatives, SI 34, SI 35 and SI 83 on SH-SY5Y human neuroblastoma cells.

SI molecules, although with different efficacy, reduced proliferation of SH-SY5Y neuroblastoma cells in a time and dose-dependent manner as assessed by MTT test and confirmed by cell counting. Proliferation assays were performed by exposing the SH-SY5Y culture to increasing concentrations of SI molecules, ranging from 1 to 10 μ M for 24, 48 and 72 h. The maximal growth inhibitory effect (75 ± 4.5 %) was reached after 72 h of exposure with SI34 10 μ M ($P < 0.001$ vs ctrl).

Evaluation of the nuclear morphology by fluorescence microscopy, analysis of DNA content by flow cytometry and determination of PARP cleavage by western blot analysis failed to demonstrate apoptosis. On the other hand, as assessed by trypan blue dye exclusion assay, exposure of SH-SY5Y cells to 10 μ M SI 34 for 24, 48 and 72 h caused a significant cytotoxic effects ($P < 0.001$ vs ctrl), indicating a necrotic cell death induced by this compound.

Evidence that SI 34 (10 μ M; 0.5-1 h) inhibited extracellular signal receptor kinase (ERK)-phosphorylation, suggests that block of MAP-kinase activation may be the mechanism by which the pyrazolo-pyrimidine derivatives exerting their antiproliferative effects in SH-SY5Y cells.

In conclusion, our data demonstrates the effectiveness of these pyrazolo-pyrimidines Src-TK inhibitors in reducing the growth of human neuroblastoma cells in vitro, indicating a promising role for these molecules as novel drugs in neuro-oncology.