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Antiproliferative effect and mechanism of action of novel triazol-containing estranes

I Zupkó[1], J Molnár[1], R Minorics[1], I Ocsovszki[2], Z Kádár[3], É Frank[3], J Wölfling[3]. *[1]University of Szeged, Department of Pharmacodynamics and Biopharmacy 6720, Hungary, [2]University of Szeged, Department of Biochemistry 6720, Hungary, [3]University of Szeged, Department of Organic Chemistry 6720, Hungary*

As a part of our systematic search for potent and selective cytostatic agents with steroidal skeleton [1-3] a set of triazol containing steroids have been synthesized and investigated in vitro using human adherent cancer cell lines (HeLa, MCF7, A431). Structure – activity relationship has been described and the most effective four estranes (1–4) out of 28 molecules were chosen for additional experiments on HeLa cells in order to characterize the mechanism of action.

Cell-cycle analysis by flow-cytometry revealed that treatment with these compounds resulted in a dose-dependent increase in subG1 population of HeLa cells. Fluorescent staining with DNA markers (Hoechst 33258 and propidium iodide) revealed nuclear condensation and disturbance in cell membrane integrity as a consequence of treatment. A treatment dependent increase in caspase-3 activity confirmed the apoptosis inducing potential of the tested agents. Crucial regulators of cell cycle and apoptosis were determined at mRNA level in order to collect information concerning the pathways involved in the cytotoxic action. Two of the most effective compounds increased the ratio of mitochondrial permeability regulating factors Bax/Bcl-2 in a concentration dependent manner. Both compounds decreased the expression of the following G2-M transition-related factors CDK1, cyclinB1, cyclinB2, cdc25B, Chk2 and p21. These results indicate that the tested agents induced apoptosis by activating intrinsic (mitochondrial) pathway. Additionally the cell cycle blockade involves ATR/Chk1 and ATM/Chk2-pathways.

Based on all of these results triazol-containing steroid skeleton is considered as promising basis for design further antiproliferative agents.

References

1. Frank É, Mucsi Z, Szécsi M, Zupkó I, Wölfling J, Schneider G: Intramolecular approach to some new D-ring-fused steroidal isoxazolidines by 1,3-dipolar cycloaddition: synthesis, theoretical and in vitro pharmacological studies. New J Chem 34: 2671-81 (2010)

2. Minorics R, Szekeres T, Krupitza G, Saiko P, Giessrigl B, Wölfling J, Frank E, Zupkó I: Antiproliferative effects of some novel synthetic solanidine analogs on HL-60 human leukemia cells in vitro. Steroids 76: 156-62 (2011)

3. Kádár Z, Molnár J, Schneider G, Zupkó I, Frank É: A facile 'click' approach to novel 15β-triazolyl-5α-androstane derivatives, and an evaluation of their antiproliferative activities in vitro. Bioorg Med Chem 20: 1396-402 (2012)

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