Abstract:

Cancer is the second leading cause of death after heart disease. Rising incidence and mortality rates are driven by resistance to existing treatments and changes in cellular signalling pathways. Therefore, the pursuit of new therapeutic strategies remains in constant and increasing demand. This research reports the synthesis and development of new hybrid anticancer molecules containing a 1,3,4-thiadiazole and a 1,3-thiazolidine-4-one heterocyclic moiety. Regarding the anticancer activity of the compounds, we found that certain compounds exhibited the most inhibitory effects on the proliferation of MCF-7 cell lines. Specifically, compounds **6e** and **9e** emerged as potential lead molecules, demonstrating more than 35- and 40-fold selectivity against MCF-7 cancer cells compared to HEK-293 cells. Additionally, the *in vitro* investigation of the anticancer mechanism revealed that compounds **6e** and **9e** induced cell cycle arrest at the G0/G1 (25.3%) and G1/S (28.2%) phases, respectively, in MCF-7 cells. Both compounds induced intracellular ROS formation, leading to cell shrinkage, chromosomal condensation, and nucleic acid damage, which suggests an apoptotic mechanism of cancer cell death. Furthermore, in Western blot analysis, compound **9e** prompted upregulation of pro-apoptotic proteins caspase-3 and -7, and downregulation of the anti-apoptotic protein BCL-2, further confirming the activation of the apoptosis pathway. Moreover, compound **9e** demonstrated potential anti-angiogenic properties in an *in vitro* tube formation assay on ACHN cells. In summary, this work suggests that the lead molecules, compounds **6e** and **9e**, may proceed to further *in vivo* antitumour studies, followed by clinical trials to thoroughly assess their anticancer efficacy, for the development of hybrid anticancer compounds.