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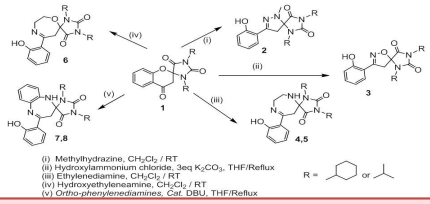
Context and Objective

Breast cancer is the first cause of cancer death in women worldwide and a major concern to public health. Targeting p53–MDM2 interaction by using appropriate molecules offers an attractive strategy for p53 activation and a promising approach for new anticancer therapies. In this context, we provide an account of the evaluation of small molecules containing oxygen and nitrogen spiro-*bisheterocycles in terms of effects on in vitro* proliferation and apoptosis of human breast cancer cell lines (MCF-7 and MDA-MB231). The wider aim is to identify novel low-molecular-weight, easily-accessible synthetic spiro compounds for cancer chemotherapy.

Experimental Procedures

- **Spiro-*bisheterocyclic* compounds 2-7 were chemically synthesized.**
- **Cell proliferation was assessed by resazurin assay.**
- **Annexin V-FITC/ PI apoptosis assays was performed in order to demonstrate apoptotic effects of spiro-*bisheterocyclic* In breast cancer cell lines.**
- **Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to analyze the wild-type *p53, MDM2, BAX and* caspase 3 gene expression levels.**



Results

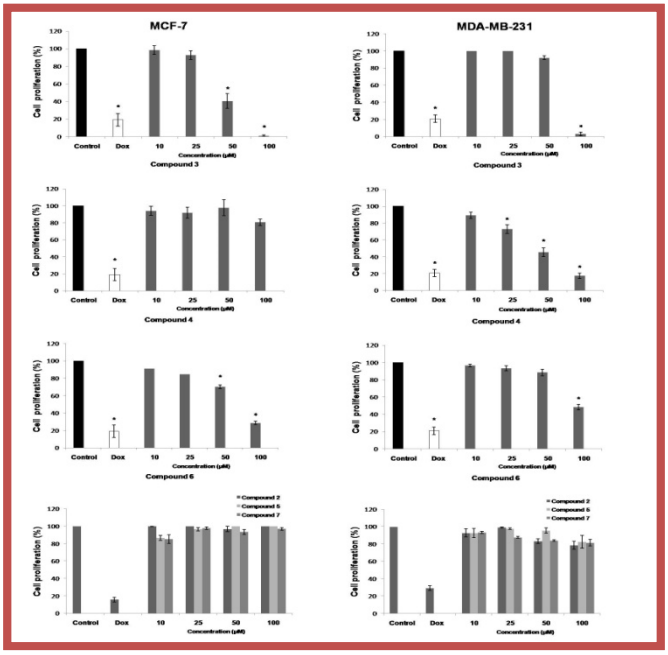


Figure 1. Effect of compounds 2-7 on the in vitro growth and proliferation of human breast cancer cells. MCF-7 and MDA-MB231 cell line were treated with 10, 25, 50 and 100 µM of compounds 2-7 for 72 h.

The treatment with compounds 3 and 6, corresponding to spiro [hydantoin-isoxazole] and spiro [hydantoin-oxazepine] respectively, resulted in a significant decrease of cell proliferation and the induction of the apoptosis in both breast cancer cell lines whereas spiro [hydantoin-diazepine] was only active against MDA-MB 231 (Figure 1).

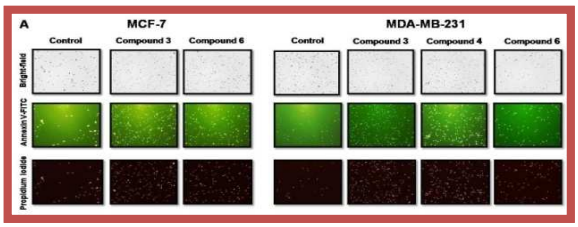


Figure 2. Drug-induced apoptosis in breast cancer cell lines MCF-7 and MDA-MB-231. Bright-field (top row), and fluorescent images of annexin V-fluorescein isothiocyanate (FITC) (middle row) and propidium iodide (PI) (bottom row) staining.

The images show significant induction of apoptosis with compounds 3 and 6 in MCF-7 cells, while strong apoptosis was observed with compounds 3, 4 and 6 in MDA-MB-231 cells (Figure 2) .

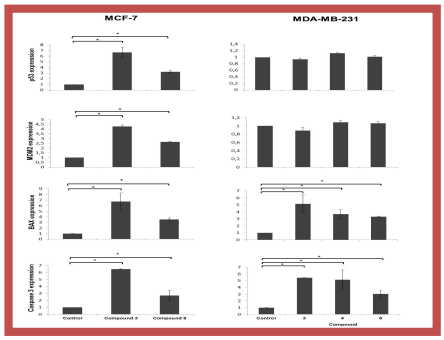


Figure 3; Results of qRT-PCR detection of p53, MDM2, BAX and caspase-3 gene expression in MCF-7 and MDA-MB-231 cells, following treatment with 100 µM of compounds 3, 4 and 6 for 72 h.

The qRT-PCR revealed an up-regulation of MDM2, strictly p53-dependent, and an increase of the expression of pro-apoptotic genes such as caspase 3 and BAX in MCF-7 wild-type p53 and MDA-MB-231 mutant p53 breast cancer cells (Figure 3).

Conclusion

In summary, this study brings the first demonstration that new small spiro-*bisheterocyclic* molecules can sensitize breast cancer cells to apoptosis by targeting p53–MDM2 interaction. We also highlighted that these spiro compounds promote apoptosis *via p53*- independent pathway(s), suggesting that these compounds represent interesting candidates as therapeutic targets for the treatment of breast cancer.