

# Proapoptotic action of a new pyrrolidinedione-thiazolidinone hybrid towards human breast carcinoma cells

Anna Bielawska<sup>1</sup>, Nataliya Finiuk<sup>2</sup>, Yuliia Kozak<sup>2</sup>, Agnieszka Gornowicz<sup>1</sup>, Robert Czarnomysy<sup>3</sup>, Roman Lesyk<sup>4</sup>,  
Krzysztof Bielawski<sup>3</sup>



<sup>1</sup>Department of Biotechnology, Medical University of Białystok, Kilinskiego 1, 15-089 Białystok, Poland

<sup>2</sup>Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology of National Academy of Sciences of Ukraine, Drahomanov  
14/16, 79005 Lviv, Ukraine

<sup>3</sup>Department of Synthesis and Technology of Drugs, Medical University of Białystok, Kilinskiego 1, 15-089 Białystok, Poland

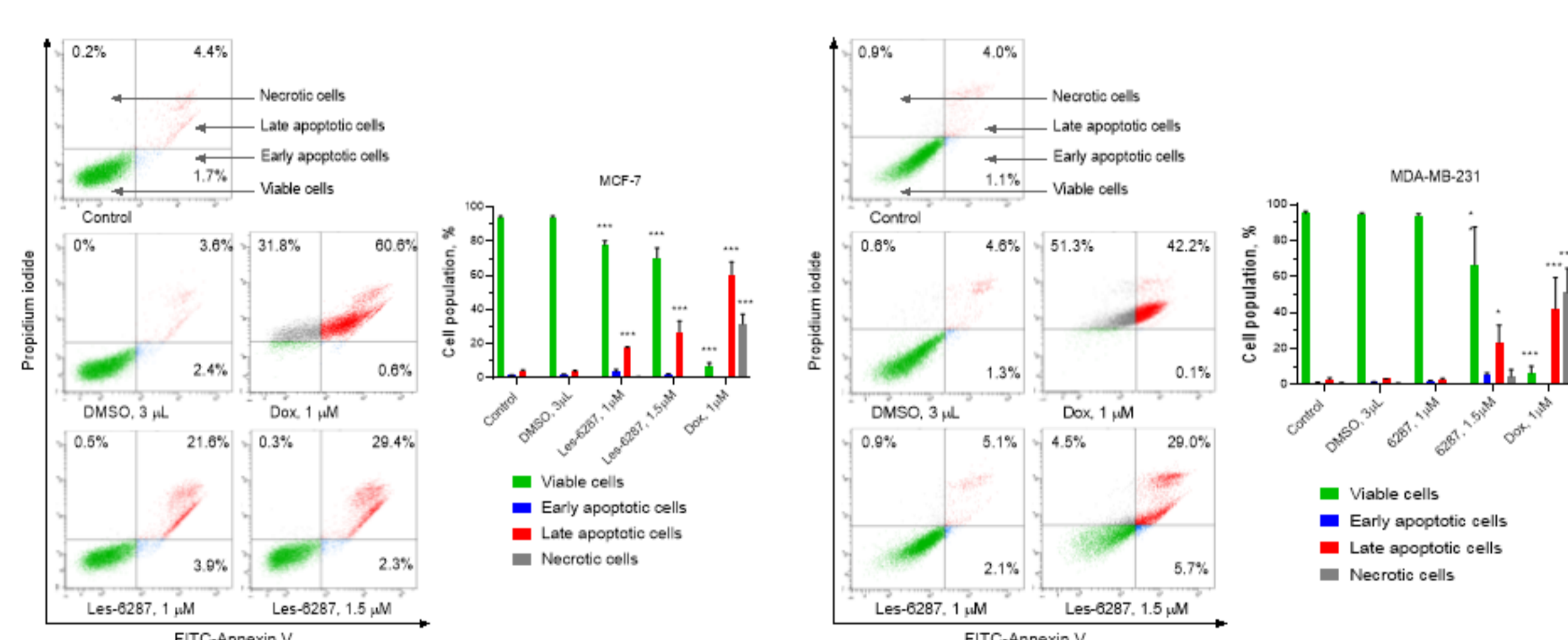
<sup>4</sup>Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv,  
Ukraine  
[anna.bielawska@umb.edu.pl](mailto:anna.bielawska@umb.edu.pl)

## INTRODUCTION

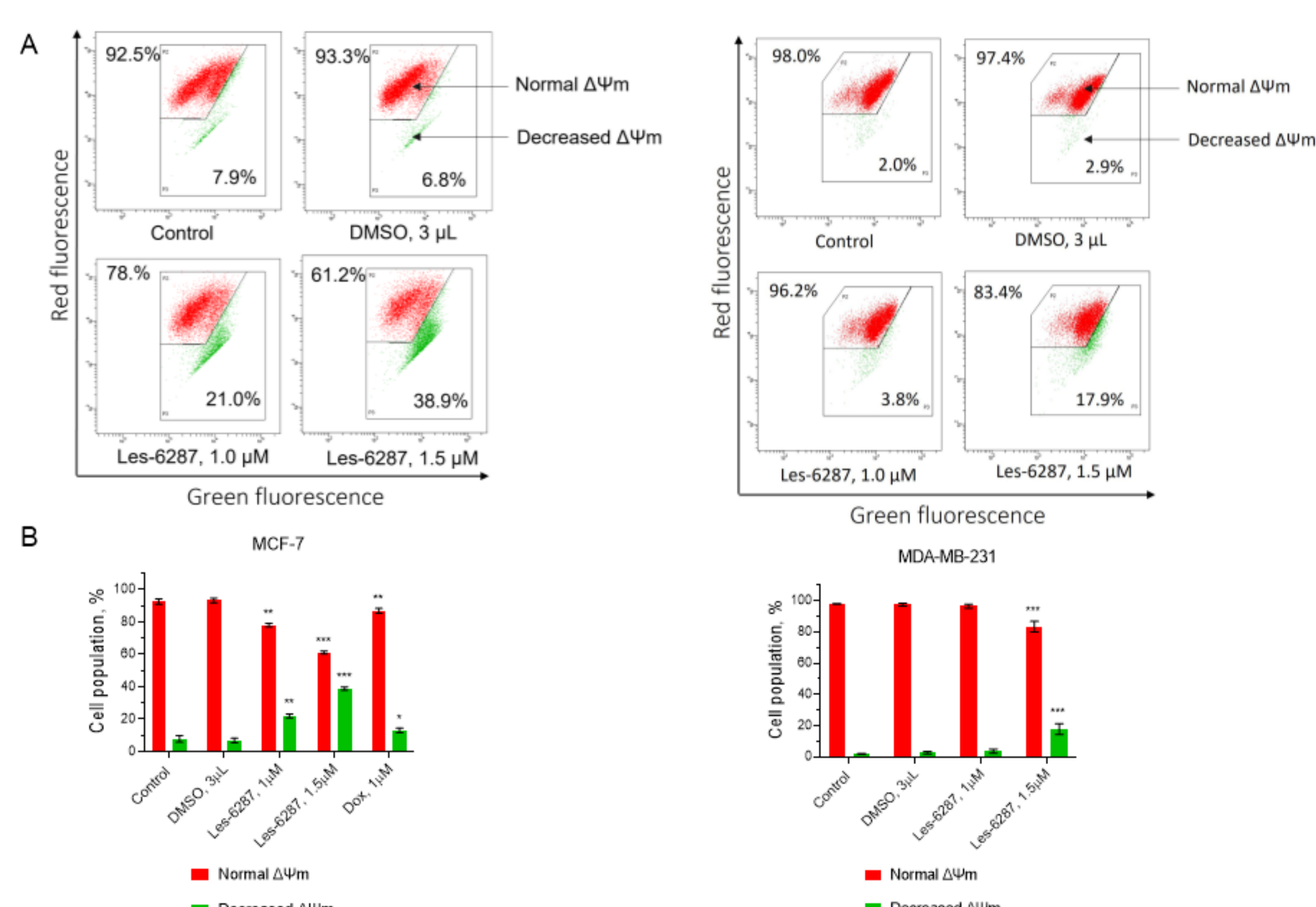
Cancer is the second worldwide cause of death. Today there are many ways to treat cancer, including surgery, chemotherapy, radiotherapy, immunotherapy, hormone and cytokine therapy, and treatment with various types of RNA molecules. Chemotherapy is still an important treatment option for malignancies. At the same time, chemotherapy is accompanied by several side effects associated with the non-specific action of the chemotherapeutic agents leading to general toxicity in the body and drug resistance. Applying the pharmacophore/molecular hybridization approaches has proven to be an effective strategy for designing and searching anticancer hit compounds with 4-thiazolidinone scaffolds. The Ciminalum and pyrrolidinedione containing molecules possess a privileged place in the design of different chemotypes of 4-thiazolidinone hybrids with anticancer activity. So, a series of pyrrolidinedione-thiazolidinone hybrid molecules with potential anticancer properties have been reported so far.

The aim of the study was to evaluate the proapoptotic action of new pyrrolidinedione-thiazolidinone hybrid (Les-6287) towards human breast carcinoma cells.

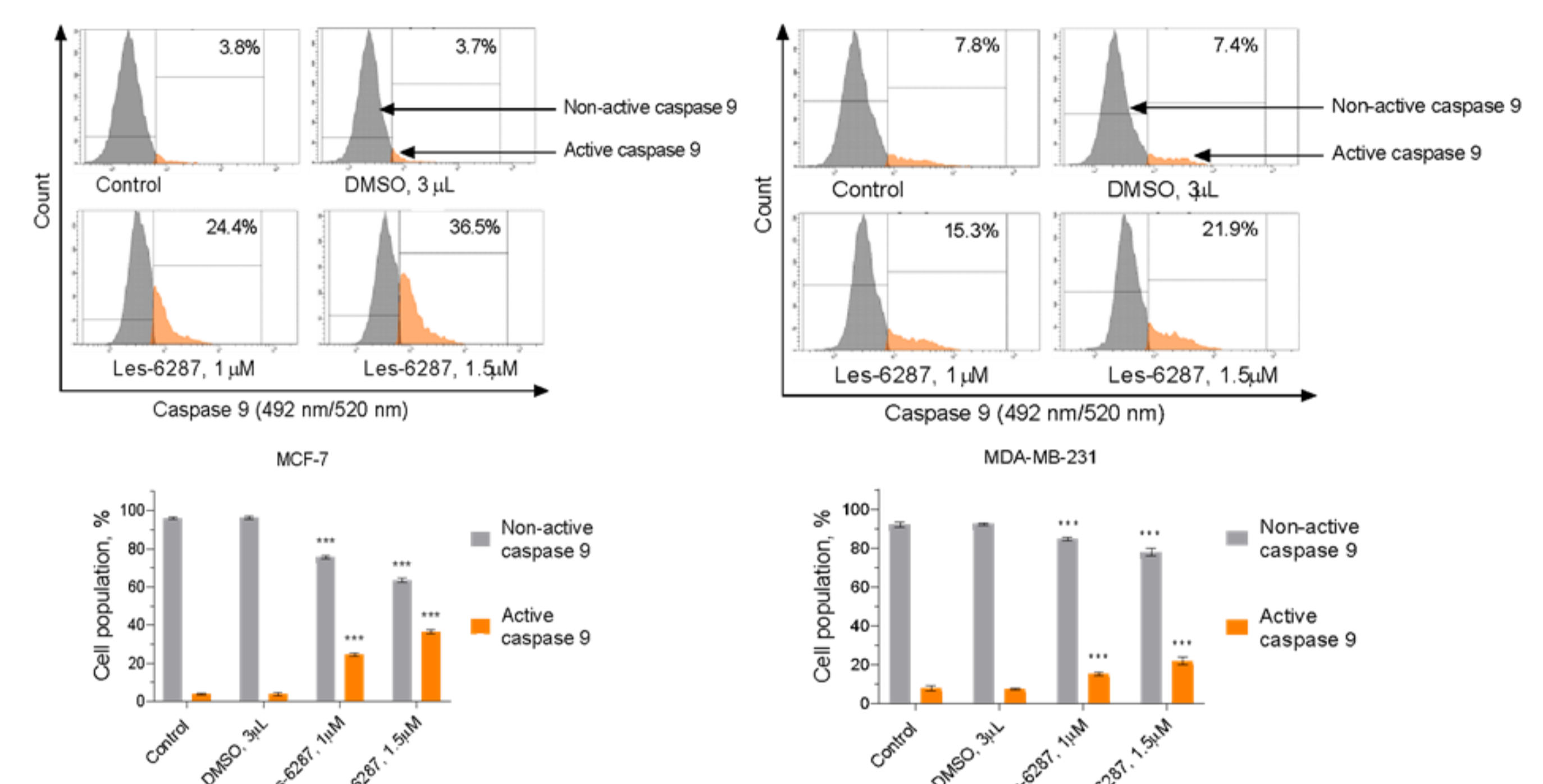
## RESULTS



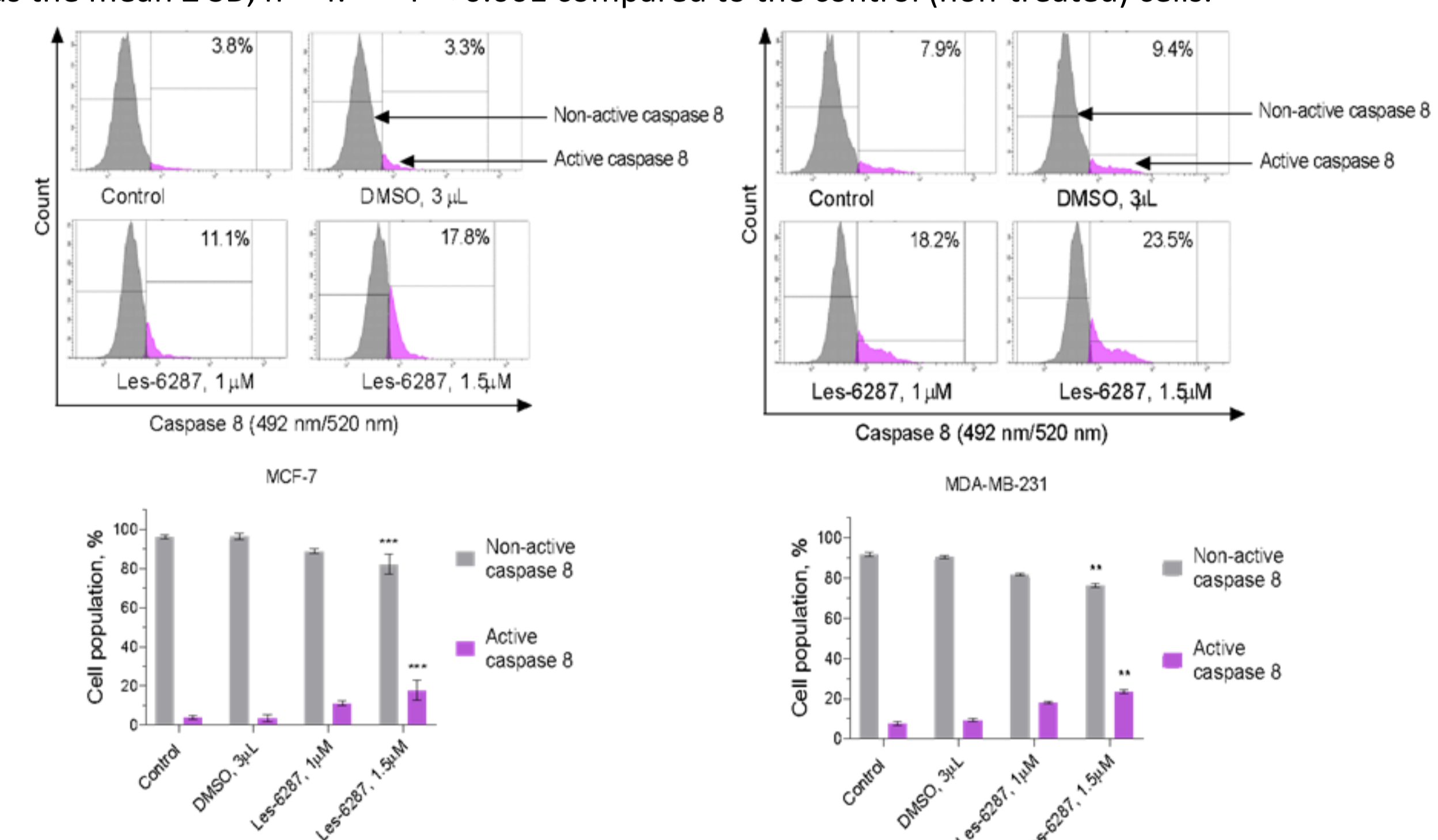
**Figure 1.** Flow cytometry analysis of human breast carcinoma MCF-7 and MDA-MB-231 cells after 24 h incubation with Les-6287 (1.0  $\mu$ M and 1.5  $\mu$ M), doxorubicin (1.0  $\mu$ M), and DMSO (0.15% corresponding the solvent concentration at 1.5  $\mu$ M of compound Les-6287) and subsequent stain-ing with Annexin V and Propidium iodide. Data are presented as the mean  $\pm$  SD, n = 4. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001 compared to the control (non-treated) cells.



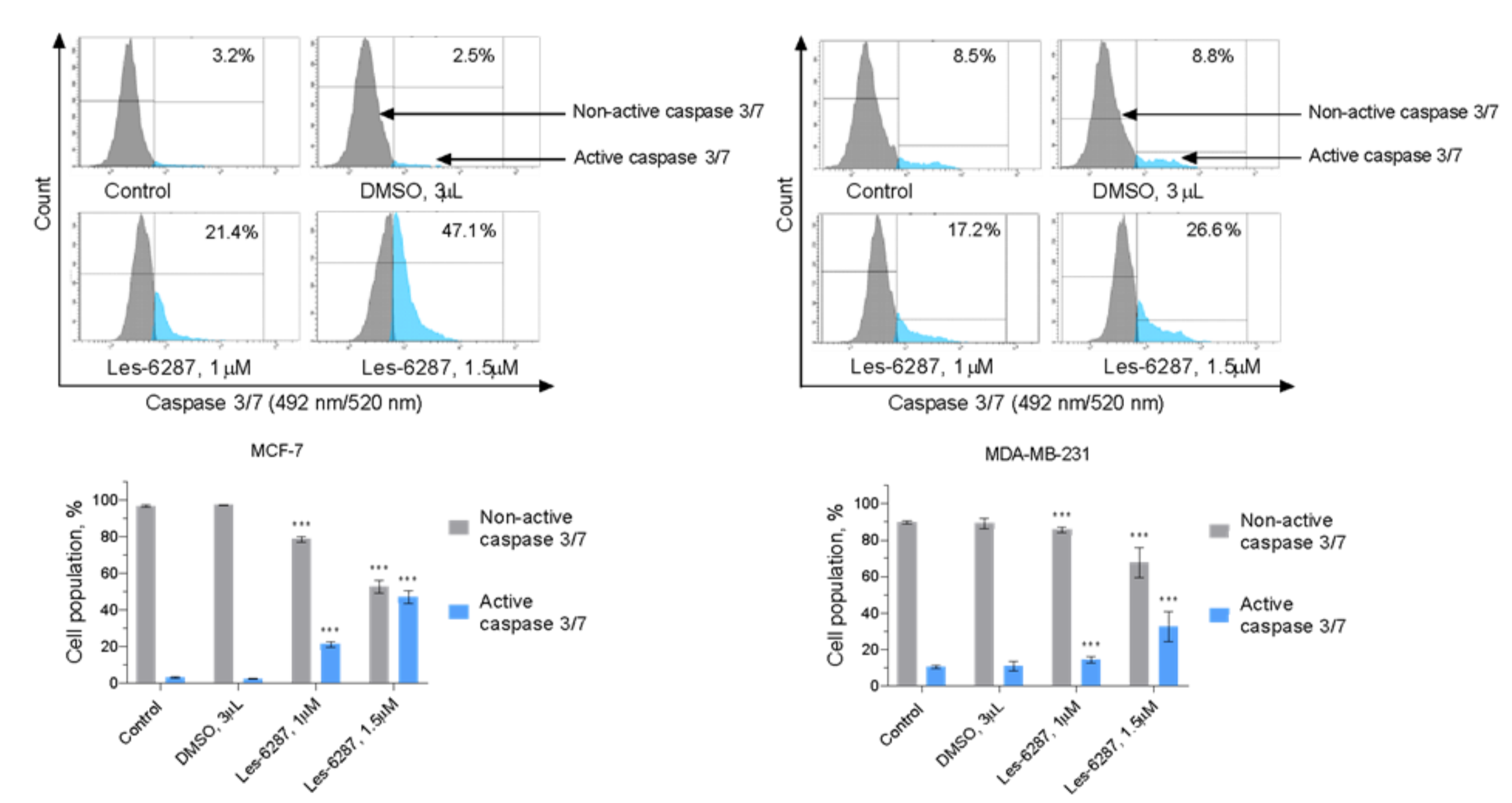
**Figure 2.** Flow cytometry analysis of mitochondrial membrane potential changes (MMP,  $\Delta\Psi_m$ ) in MCF-7 and MDA-MB-231 breast cancer cells after 24 h incubation with Les-6287 (1.0  $\mu$ M and 1.5  $\mu$ M), doxorubicin (1.0  $\mu$ M), and DMSO (0.15% corresponding the solvent concentration at 1.5  $\mu$ M of compound Les-6287). Data are presented as the mean  $\pm$  SD, n = 4. \*\*\*P < 0.001 compared to the control (non-treated) cells.



**Figure 3.** Flow cytometry analysis of caspase 9 activity in MCF-7 and MDA-MB-231 breast cancer cells after 24 h incubation with Les-6287 (1.0  $\mu$ M and 1.5  $\mu$ M), doxorubicin (1.0  $\mu$ M), and DMSO (0.15% corresponding to the solvent concentration at 1.5  $\mu$ M of compound Les-6287). Data are presented as the mean  $\pm$  SD, n = 4. \*\*\*P < 0.001 compared to the control (non-treated) cells.



**Figure 4.** Flow cytometry analysis of caspase 8 activity in MCF-7 and MDA-MB-231 breast cancer cells after 24 h incubation with Les-6287 (1.0  $\mu$ M and 1.5  $\mu$ M), doxorubicin (1.0  $\mu$ M), and DMSO (0.15% corresponding to the solvent concentration at 1.5  $\mu$ M of compound Les-6287). Data are presented as the mean  $\pm$  SD, n = 4. \*\*P < 0.01; \*\*\*P < 0.001 compared to the control (non-treated) cells.



**Figure 5.** Flow cytometry analysis of caspase 3/7 activity in MCF-7 and MDA-MB-231 breast cancer cells after 24 h incubation with Les-6287 (1.0  $\mu$ M and 1.5  $\mu$ M) and DMSO (0.15% corresponding to the solvent concentration at 1.5  $\mu$ M of compound Les-6287). Data are presented as the mean  $\pm$  SD, n = 4. \*\*\*P < 0.001 compared to the control (non-treated) cells.

## CONCLUSIONS

We have estimated the effect of the Les-6287 on the induction of the apoptotic in MCF-7 and MDA-MB-231 cells after 24 h of exposure. The double staining of cells with annexin V-FITC (AV) and propidium iodide (PI) as well as mitochondrial membrane potential analysis was used for this purpose as well as activity of caspase-8, caspase-9 and caspase-3/7 was checked by flow cytometry.

We proved that the Les-6287 derivative induces apoptosis using extrinsic and intrinsic pathways via a decrease of the membrane potential, increasing the activity of caspase 3/7, 8, 9 in all immunohistochemically different human breast cancer cells.