Evaluation of the proapoptotic activity of Novel Platinum(II) bis(thiosemicarbazone) Complex in Breast Cancer Cells

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INTRODUCTION

Disturbing data on cancer incidence, as well as the increasing resistance and high toxicity of conventionally used anticancer drugs, are major obstacles to effective chemotherapy. Overcoming these problems and creating new, more effective chemotherapeutics that spare normal tissues simultaneously is the challenge of modern oncology. Hydrazine derivatives such as thiosemicarbazone and its analogs may provide a solution to the above problems. These compounds, through the presence of nitrogen and sulfur atoms in their structure, have donor sites that can link to a metal ion to form complexes. It is through their ability to complex that these compounds provide a wonderful framework for developing new, selective compounds with promising biological properties depending on the metal used in their structure.

Therefore, the present work aimed to synthesize novel bis(thiosemicarbazone) derivative based on platinum (thioPt) in the core of the complex and evaluate its anticancer properties against MCF-7 and MDA-MB-231 breast cancer cells.

METHODS

The cytotoxic activity of the novel compounds was examined using the MTT method of Carmichael. Evaluation of apoptosis induction was done with the Annexin V/propidium iodide assay. Moreover, using the flow cytometer, the effects of the tested compounds on mitochondrial potential change were assessed and caspase-8 and -9 activity.

CONCLUSION

The results of our study showed that the analyzed compounds: novel ligand (thio) and a Pt(II) bis(thiosemicarbazone) complex (thioPt) (Figure 1 and 2) exert inhibitory effects on the viability of breast cancer cells (MCF-7, MDA-MB-231). Furthermore, the novel thioPt complex was less toxic towards MCF-10A normal breast epithelial cells (Figure 3).

By investigating the mechanism of action of the novel complex at the cellular level, thioPt has been shown to be capable of inducing apoptosis in MCF-7 and MDA-MB-231 breast cancer cells (Figure 4). Further flow cytometer experiments showed that thioPt potently induce apoptosis through two pathways, an intrinsic pathway with a sharp loss of mitochondrial membrane potential (Figure 5) and an extrinsic pathway where an increase in caspase-8 activity was observed (Figure 6), ultimately leading to an increase in active caspase 3/7 levels (Figure 7).

RESULTS

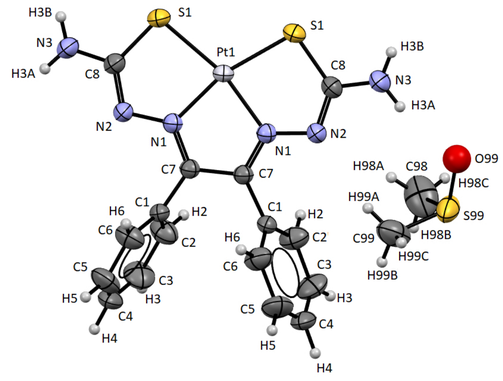

Figure 1. Crystal structure of thioPt x DMSO with the atom labelling scheme.

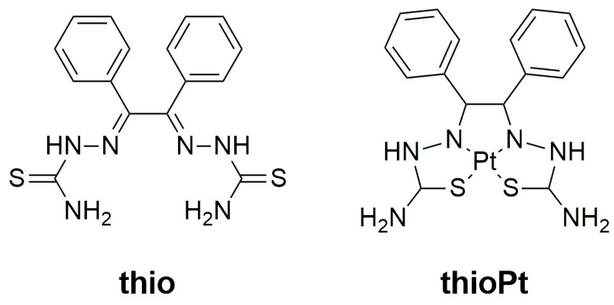

Figure 2. Chemical structures of tested compounds (**thio** ligand and **thioPt** complex).

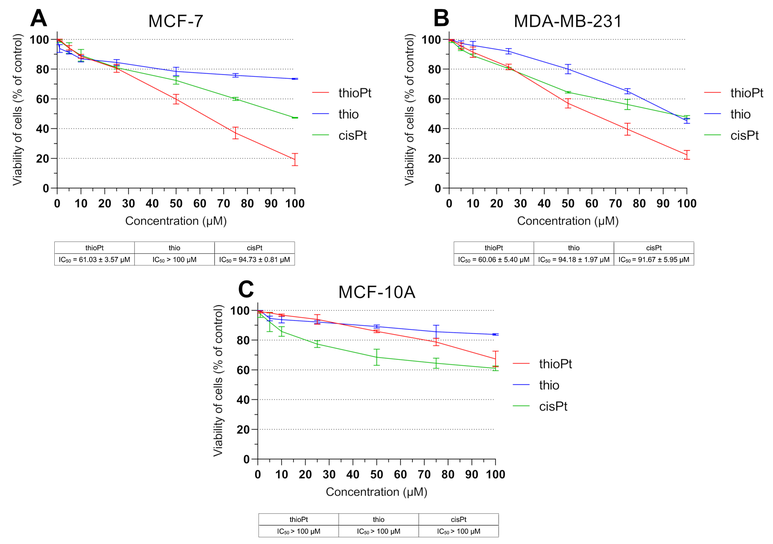

Figure 3. Viability of MCF-7 (A), MDA-MB-231 (B) breast cancer cells and MCF-10A (C) normal breast ephitelial cells treated for 24 h with different concentrations of the tested compounds. Mean values ±SD from three independent experiments (n = 3) done in duplicate are presented.

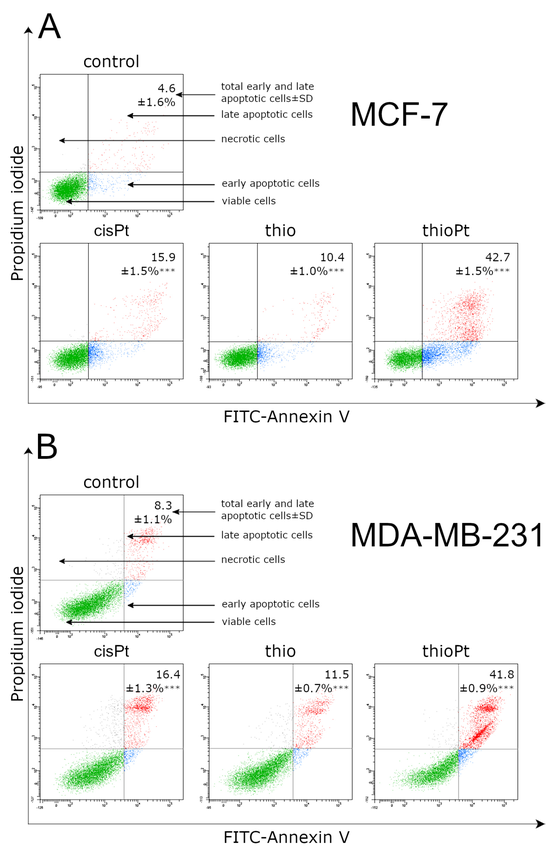

Figure 4. Flow cytometry analysis of MCF-7 (A) and MDA-MB-231 (B) cells after 24 h incubation with tested compounds (50 μM) and subsequent staining with Annexin V and propidium iodide. Mean values ±SD from three independent experiments (n = 3) done in duplicate are presented.*** p < 0.001 vs. control group.

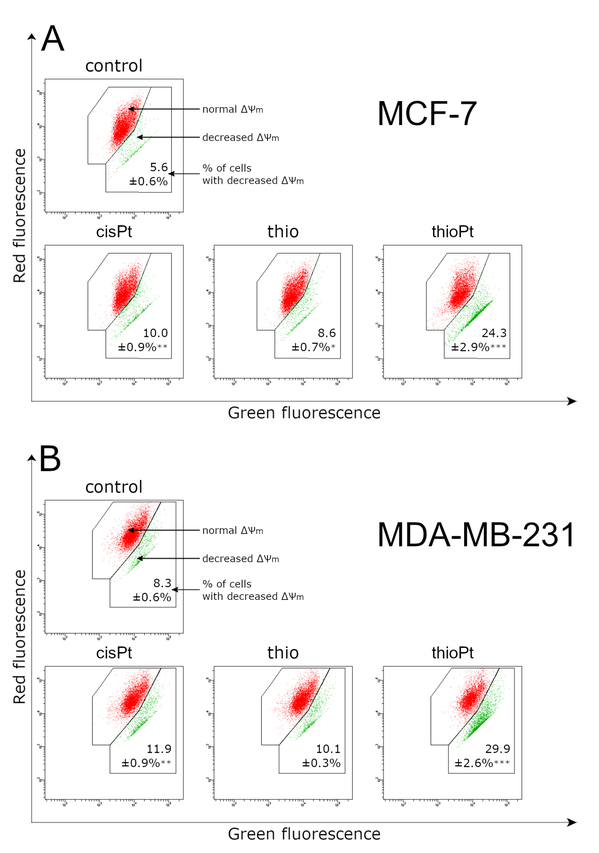

Figure 5. Fluorescence of MCF-7 (A) and MDA-MB-231 (B) cells treated for 24 h with tested compunds (50 μM) incubated with mitochondrial membrane potential probe JC-1. Mean percentage values from 3 independent experiments (n = 3) done in duplicate are presented.* p < 0.05 vs. control group, ** p < 0.01 vs. control group, *** p < 0.001 vs. control group.

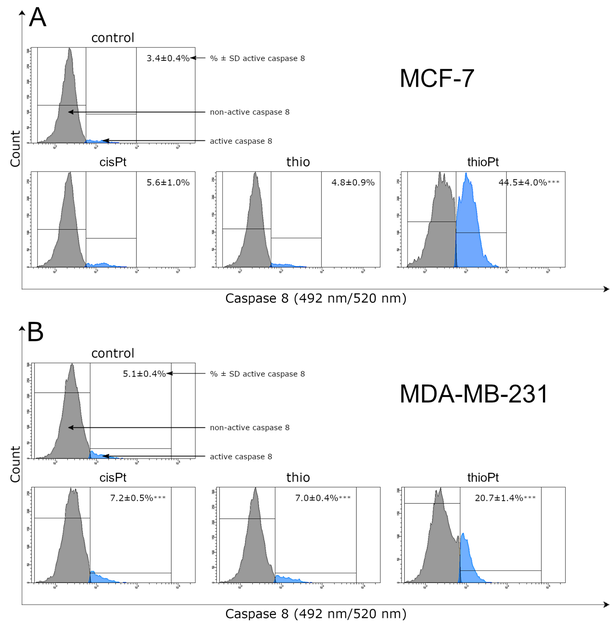

Figure 6. Flow cytometry analysis of caspase 8 activity in MCF-7 (A) and MDA-MB-231 (B) cells after 24 h incubation with tested compunds. Mean values ±SD from three independent experiments (n = 3) done in duplicate are presented. *** p < 0.001 vs. control group.

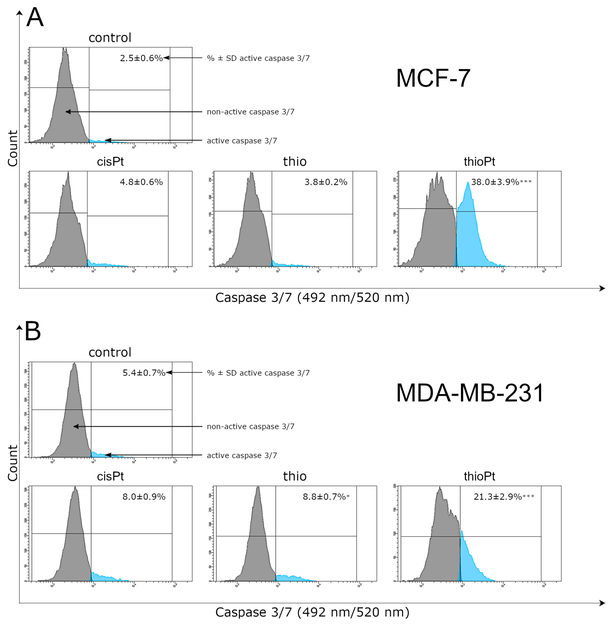

Figure 7. Flow cytometry analysis of caspase 3/7 activity in MCF-7 (A) and MDA-MB-231 (B) cells after 24 h incubation with tested compunds. Mean values ±SD from three independent experiments (n = 3) done in duplicate are presented. * p < 0.05 vs. control group, *** p < 0.001 vs. control group.