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Pro-apoptotic and anti-migration properties of a thiazoline-containing platinum(II) complex in MDA-MB-231 breast cancer cells: role of melatonin as synergistic agent

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Samuel Estirado[1*], Elena Fernández-Delgado[1*], Emilio Viñuelas-Zahínos[2], Francisco Luna-Giles[2], Ana B. Rodríguez[1], José A. Pariente[1] and Javier Espino[1]

[1] Department of Physiology (Neuroimmunophysiology and Chrononutrition Research Group), Faculty of Science, University of Extremadura;
[2] Department of Organic and Inorganic Chemistry (Chemistry of Coordination Group), Faculty of Science, University of Extremadura

* Corresponding author: estirado@unex.es, elenafd@unex.es

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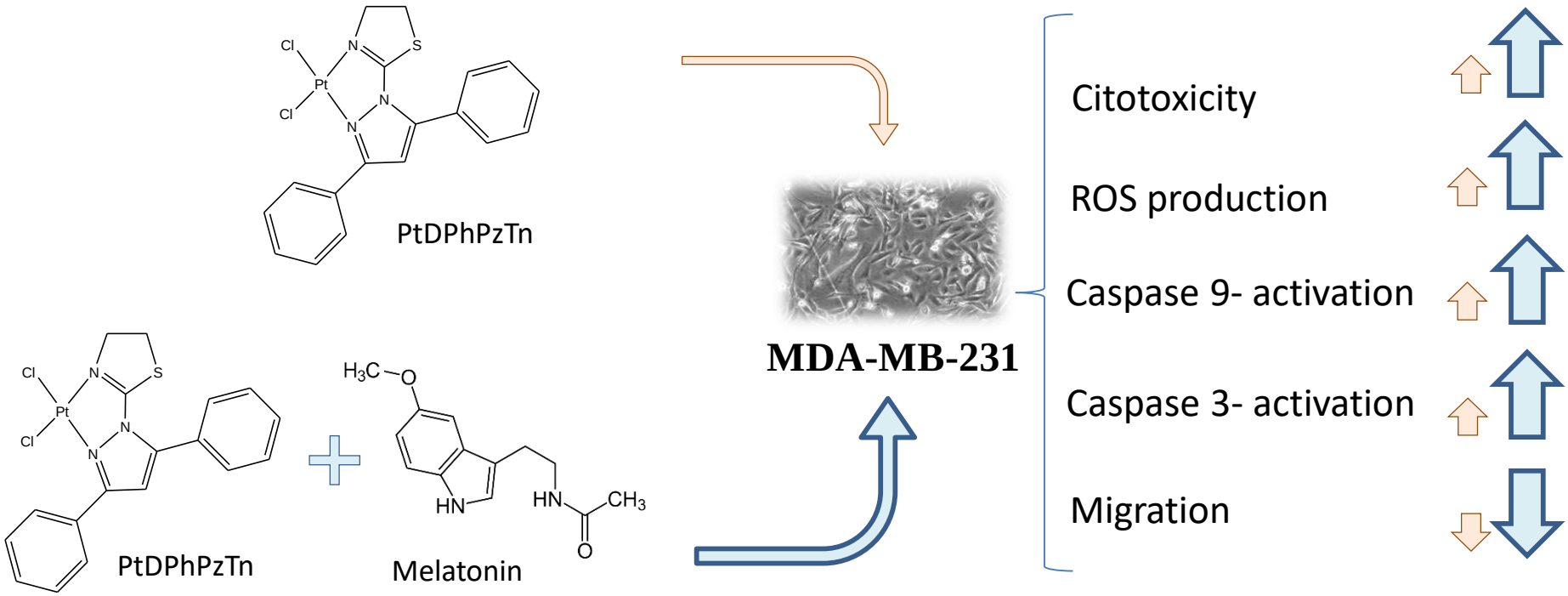
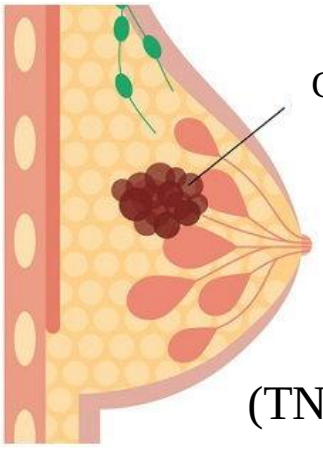
Graphical Abstract



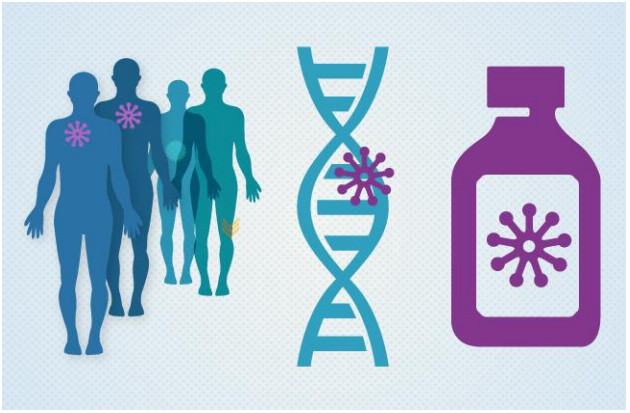
Abstract:
Triple-negative breast cancer (TNBC) is an aggressive cancer that does not respond to hormonal and HER2-targeted therapies and have poor prognosis. Therefore, there is a need for the development of convenient anticancer strategies that can be effectively used for the treatment of TNBC. Herein, we evaluated the antitumoral potential of a platinum(II) complex coordinated with the ligand 2-(3,5-diphenylpyrazol-1-yl)-2-thiazoline (DPhPzTn), hereafter PtDPhPzTn, against the TNBC cell line MDA-MB-231. The putative potentiating actions of melatonin on the tumor-killing ability of PtDPhPzTn were also checked in MDA-MB-231 cells. We first examined the cytotoxic effect of both PtDPhPzTn and melatonin in the TNBC cell line, which were dose-dependent. We then combined different doses of the PtDPhPzTn and melatonin to test their combinatorial effect and found a synergistic effect, especially when combining 1 mM melatonin and 5, 10 and 25 µM PtDPhPzTn. Additionally, PtDPhPzTn induced apoptosis mediated by caspase-3 and -9 activation and dependent on reactive oxygen species overproduction. Likewise, PtDPhPzTn almost completely blunted the migration capacity of MDA-MB-231 cells. Combined treatment with PtDPhPzTn and melatonin moderately potentiated the pro-apoptotic and anti-migratory actions of the complex. Therefore, these findings suggest that aromatic groups improve the cytotoxicity of the compound and provide evidence that PtDPhPzTn and melatonin could be potentially applied to TBNC treatment as synergistic agents.

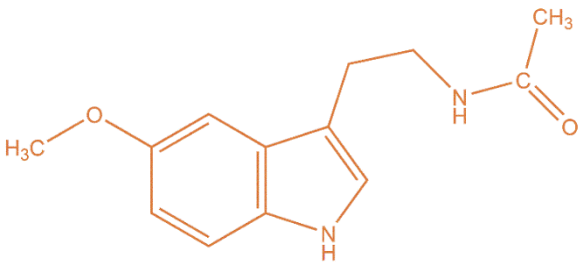
Keywords: Breast cancer, Caspases, Cytotoxicity, Melatonin, Platinum(II) complex

Introduction



(TNBC)

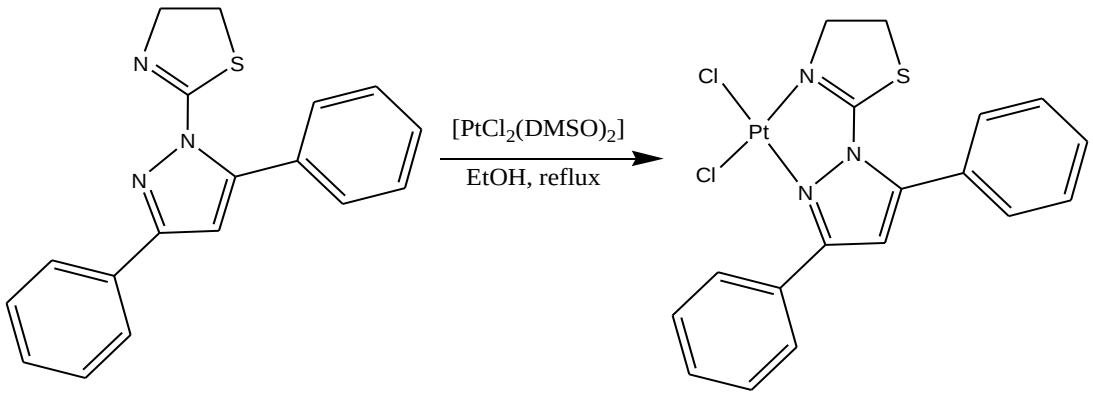




Tumour killing ability

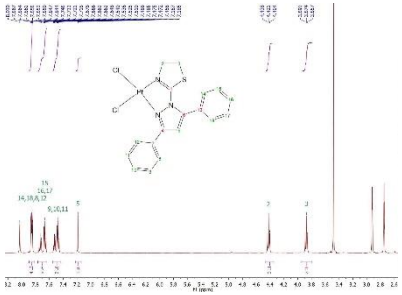
Results and discussion

Synthesis:

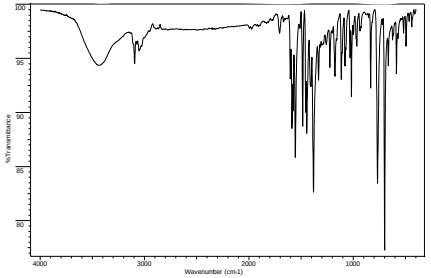


Characterization:

^{1}H NMR spectroscopy

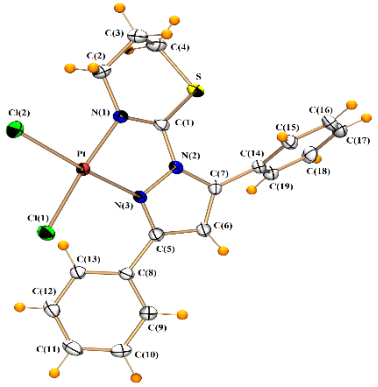


IR spectroscopy



Elemental analysis

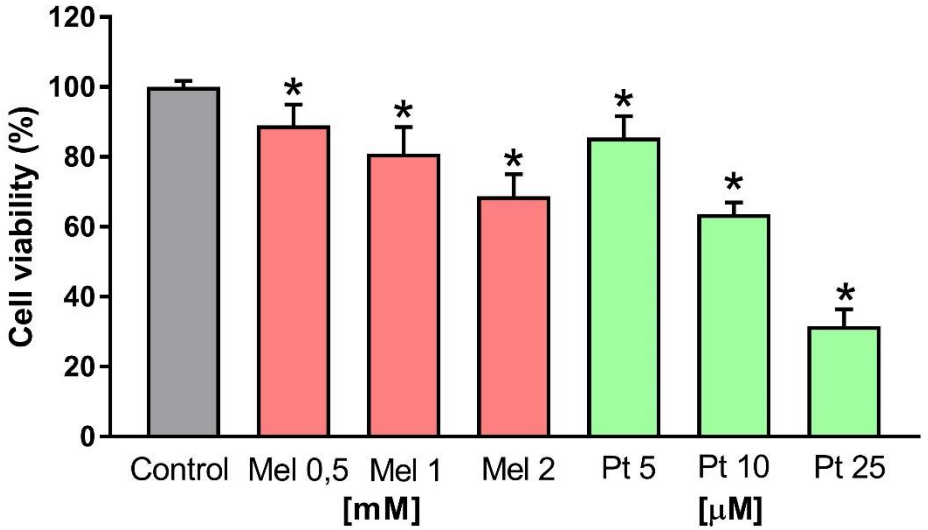
Single cristal X-ray analysis



Results and discussion

Viability:

Dose-response curve of the thiazoline-containing Pt(II) complex and melatonin on cell viability of **MDA-MB-231** cells after 24h of treatment



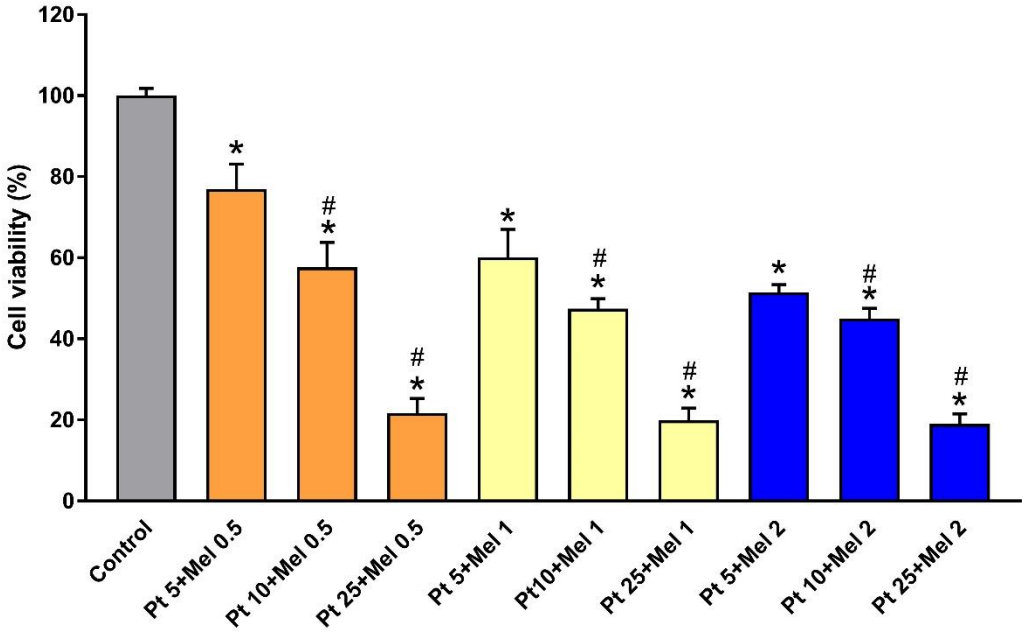
IC_{50}:
- 10.4 μM PtDPhPzTn
- 4.3 mM melatonin

Values are presented as means ± SD of 5 separate experiments and expressed as percentage of control values (DMF-treated samples).
*P < 0.05 compared to control values.

Results and discussion

In vitro citotoxicity assay:

Combination of different doses of the thiazoline-containing Pt(II) complex and melatonin on cell viability of **MDA-MB-231** cells after 24h of treatment.



Treatments:
- [µM] PtDPhPzTn
- [mM] melatonin

Values represent means ± S.D. of 5 independent experiments. *P < 0.05 compared to control values. #P < 0.05 compared to its corresponding Pt 5+Mel X values.

Results and discussion

Synergistic effect of the combination of PtDPhPzTn and melatonin on cell viability of **MDA-MB-231** cells after 24h of treatment.

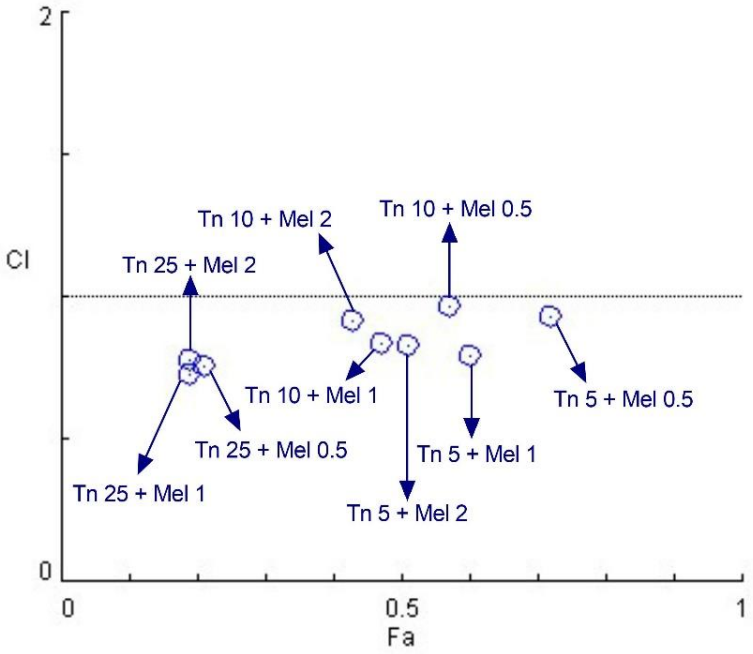
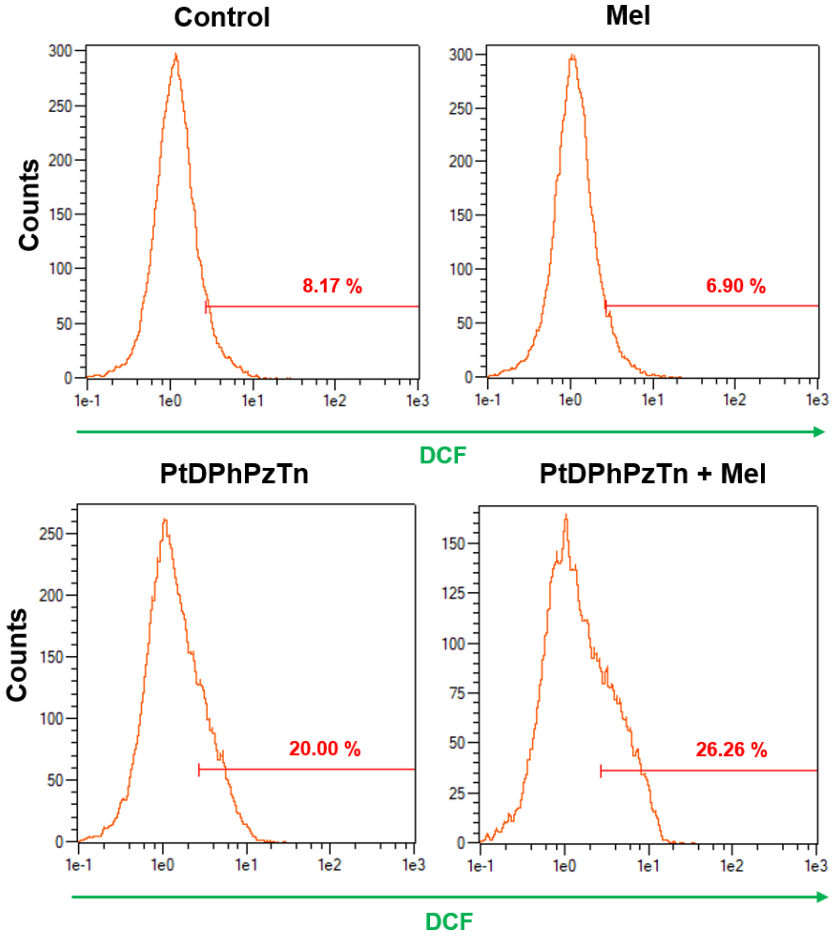


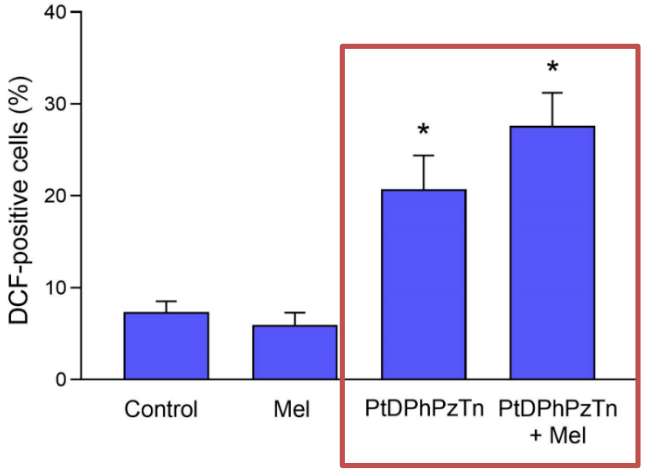
Table I: Calculation of the combination index (CI) data for non-constant combination of PtDPhPzTn and melatonin in cell death induction.

PtDPhPzTn (µM)	Melatonin (mM)	Effect (Fa)	CI
5	0.5	0.72	0.93
10	0.5	0.57	0.96
25	0.5	0.21	0.75
5	1	0.60	0.79
10	1	0.47	0.83
25	1	0.19	0.72
5	2	0.51	0.83
10	2	0.45	0.91
25	2	0.19	0.77

Results and discussion

Determination of reactive oxygen species (ROS) production :





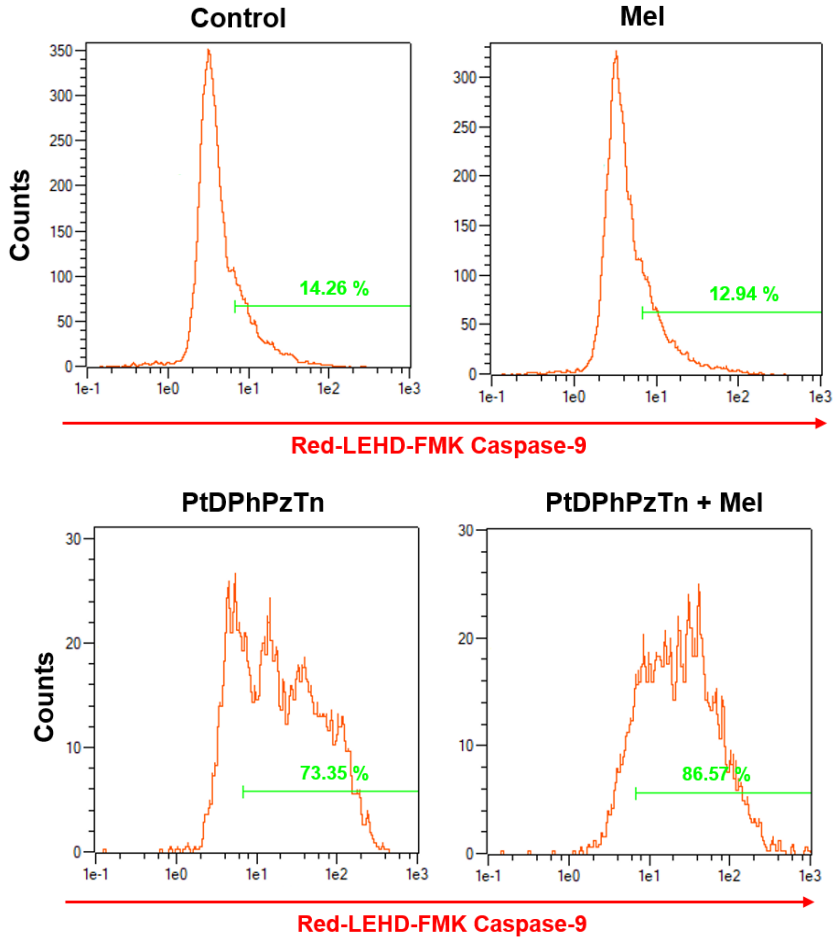
Values represent means ± S.D. of 5 independent experiments. *P < 0.05 compared to control.

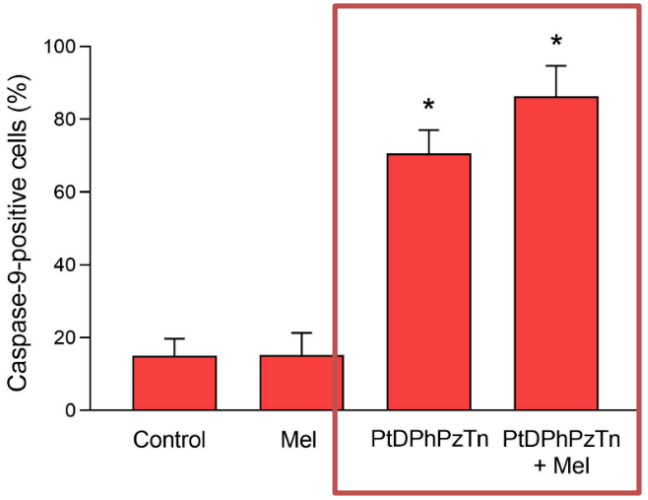
Treatments (4 h):

- 1 mM melatonin
- 10.4 µM PtDPhPzTn
- 10.4 µM PtDPhPzTn + 1 mM melatonin

Results and discussion

Determination of caspase-9 activation:





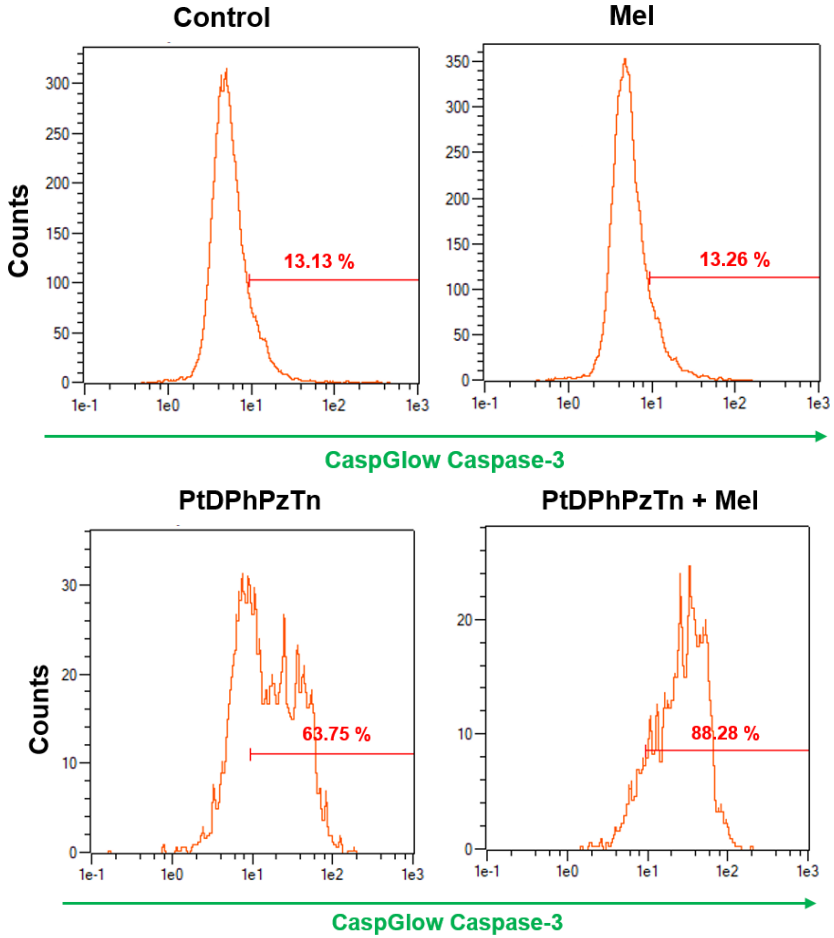
Values represent means ± S.D. of 5 independent experiments. *P < 0.05 compared to control.

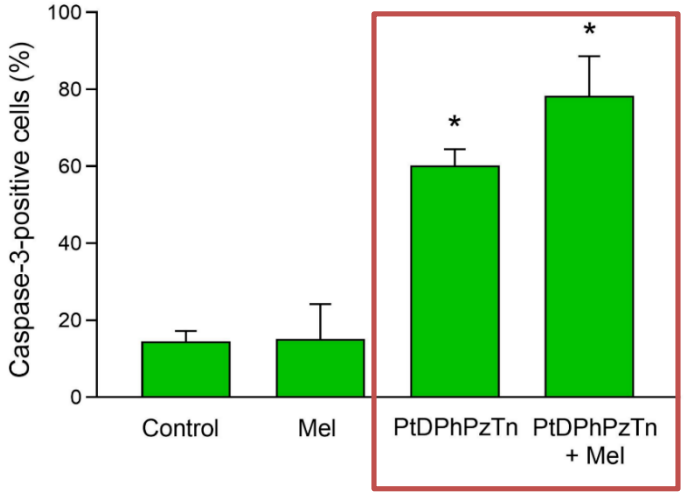
Treatments (24 h):

- 1 mM melatonin
- 10.4 µM PtDPhPzTn
- 10.4 µM PtDPhPzTn + 1 mM melatonin

Results and discussion

Determination of caspase-3 activation:





Values represent means ± S.D. of 5 independent experiments. *P < 0.05 compared to control.

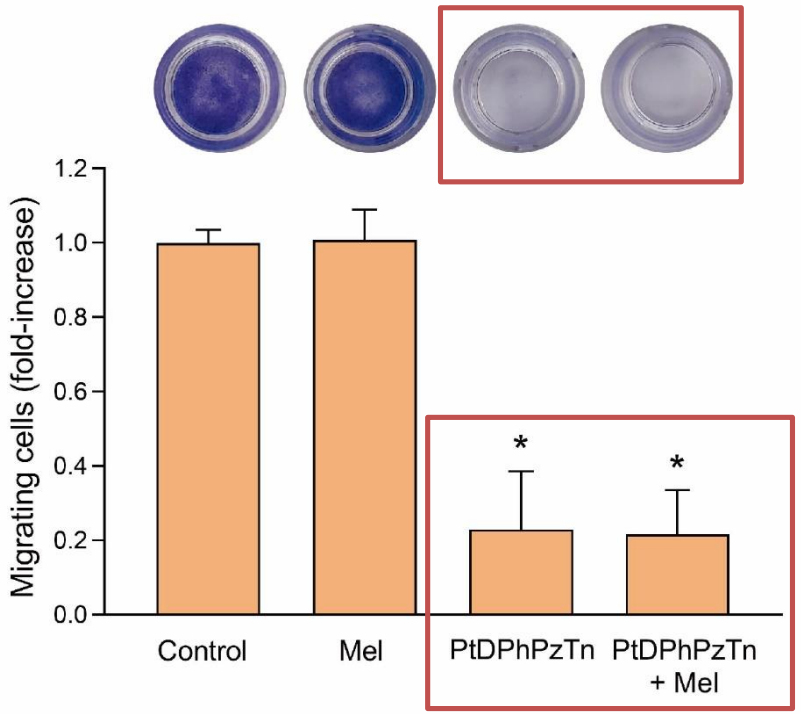
Treatments (24 h):

- 1 mM melatonin
- 10.4 µM PtDPhPzTn
- 10.4 µM PtDPhPzTn + 1 mM melatonin

Results and discussion

Transwell migration assay:

Effect of combinatory treatment of melatonin and PtDPhPzTn on migration properties of **MDA-MB-231** cells after 24h of treatment.



Treatments (24 h):

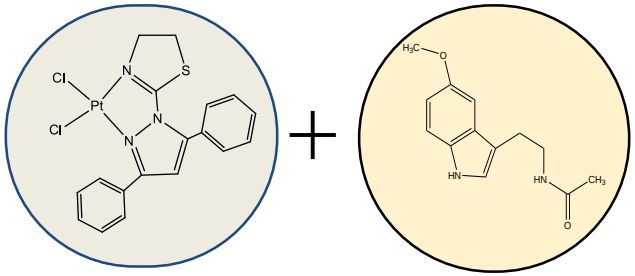
- 1 mM melatonin
- 10.4 µM PtDPhPzTn
- 10.4 µM PtDPhPzTn + 1 mM melatonin

Values are presented as means ± SD of 5 separate experiments and expressed as percentage of control values (DMF-treated samples).
*P < 0.05 compared to control values.

Conclusions

PtDPhPzTn induced apoptosis mediated by **caspase-3** and **-9** activation and dependent on **reactive oxygen species** overproduction.

The **anti-proliferative** and **pro-oxidant** properties of **melatonin** potentiated the **tumor-killing ability of PtDPhPzTn** in MDA-MB-231 cells.



PtDPhPzTn + Melatonin

- Citotoxicity ↑
- ROS production ↑
- Caspase 9- activation ↑
- Caspase 3- activation ↑
- Migration ↓

Acknowledgments

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