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New ruthenium-cyclopentadienyl agents as a new strategy to fight colorectal cancer

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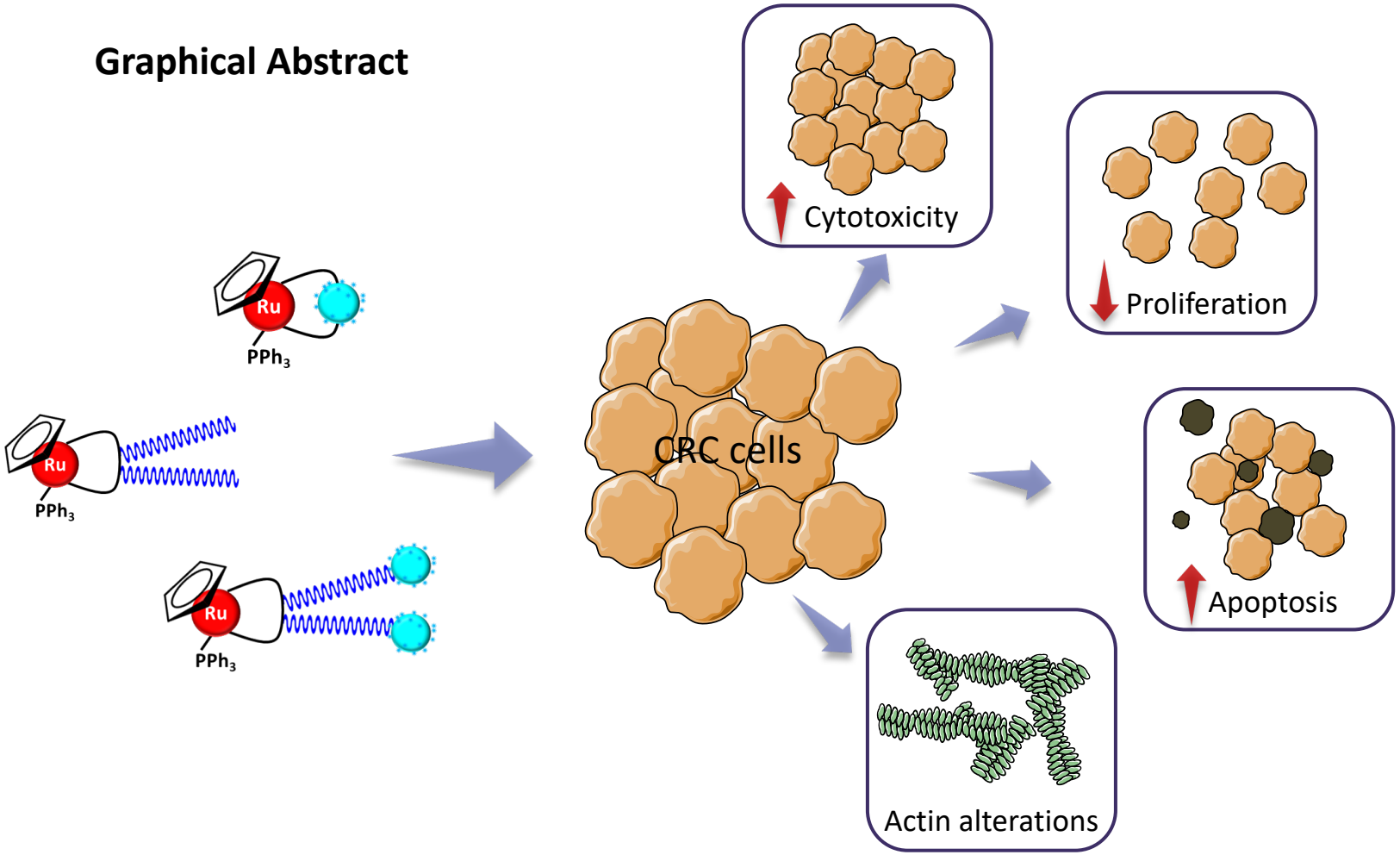
New ruthenium-cyclopentadienyl agents as a new strategy to fight colorectal cancer

Graphical Abstract



Abstract

Colorectal cancer (CRC) is one of the most lethal cancers worldwide, however it has limited chemotherapeutic agents available. CRC harboring KRAS and BRAF mutations are correlated with resistance to EGFR inhibitors what constitutes a relevant clinical problem. Ruthenium (Ru) drugs had arisen as one of the most promising metallodrugs with features that increase their specificity and selectivity toward cancer cells.

Recently, a new family of Ru-cyclopentadienyl conjugates was designed using macromolecules and/or biomolecules. Here, we aimed to study the effect of these new Ru conjugates in CRC cells in order to study the potential increase in selectivity and efficiency in CRC cells. In this work, we used two CRC-derived cell lines with KRAS and BRAF mutations and a normal colon cell line to study cellular cytotoxicity, antiproliferative activity, cell death mechanism, intracellular distribution, MAPK-ERK and PI3K-AKT signaling pathways and actin cytoskeleton effects of the compounds.

Our results revealed that Ru agents are more cytotoxic for CRC cells, induce cell cycle arrest, decrease the ability of cells to proliferate, induce apoptosis and preferentially localize in membrane and cytoskeleton of CRC cells. Ru agents also affect F-actin polymerization and MAPK-ERK and PI3K-AKT signaling pathways.

Overall, our results showed that Ru compounds present promising anticancer activity in CRC cells, mainly in KRAS mutated cell lines, what could bring new avenues in CRC therapy.

Keywords: Colorectal cancer; KRAS; BRAF; Ruthenium agents

Introduction

Colorectal cancer

- Colorectal cancer is the 3rd type of cancer most incident.
- Colorectal cancer is the 2nd leading cause of cancer death.

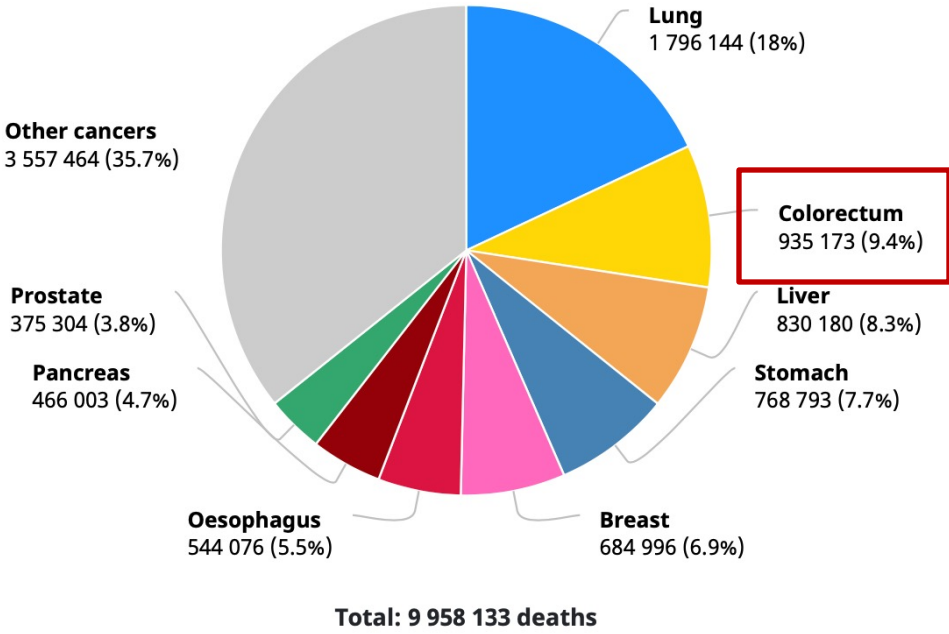


Figure 1. Number of global deaths of CRC in GLOBOCAN 2020. Adapted from: Sung et al., 2021.

Introduction

Colorectal cancer carcinogenesis

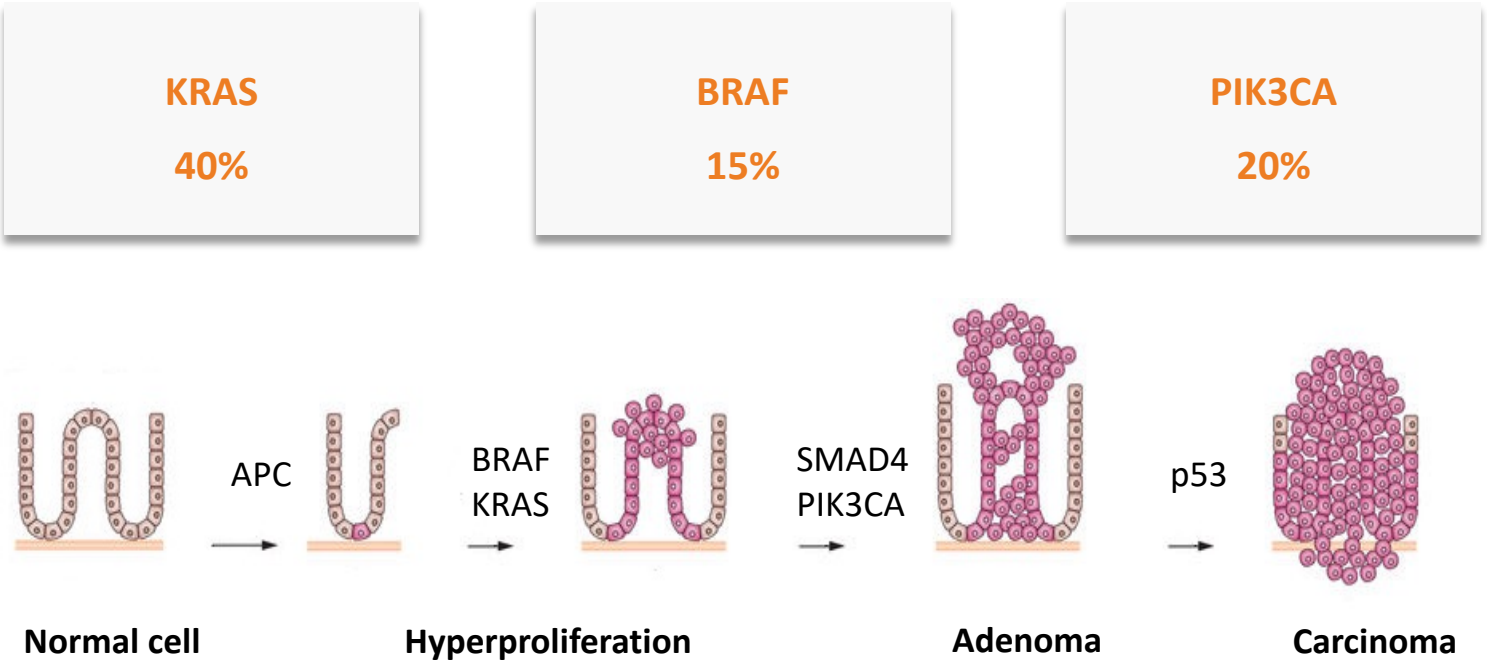


Figure 2. CRC carcinogenesis. Adapted from: Kuipers, 2015

Introduction

Colorectal cancer therapy

Surgery

Chemotherapy 

5-Fluorouracil

The most widely used chemotherapy agent to treat CRC

- **Success rate as low** as 10-15%
- Severe **side effects and resistance**

Targeted therapies

Target growth factor pathways in CRC- EGFR inhibitors

- CRC with KRAS, BRAF and PIK3CA mutations **do not respond to EGFR inhibitors**

Introduction

Colorectal cancer therapy

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Clinical relevant problem that needs to be overcome!

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Target growth factor pathways in CRC- EGFR inhibitors

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Introduction

Ruthenium drugs

- **Low toxicity**
- **Potential to overcome platinum-resistance**
- **Activation-by-reduction**
- **Low ligand-exchange rate**

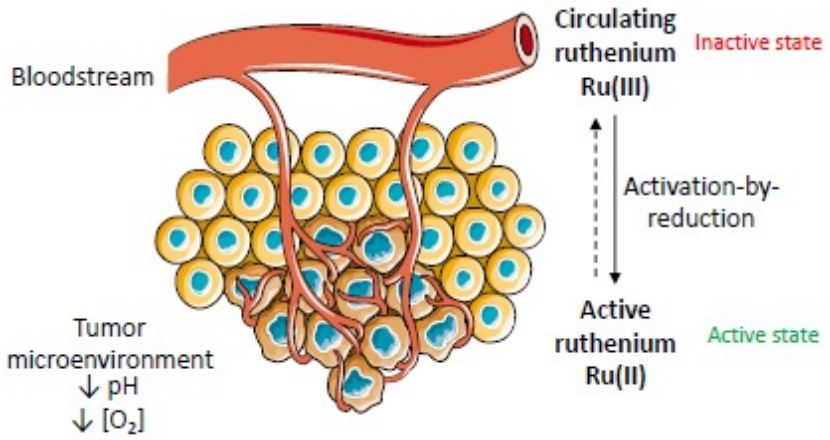
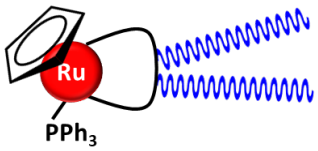


Figure 3. "Activation-by-reduction" mechanism of ruthenium(III) complexes. Adapted from: Antonarakis & Emadi, 2010

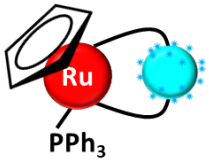
Introduction

New ruthenium-cyclopentadienyl agents

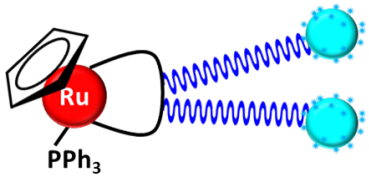
Compound 1



Compound 2



Compound 3



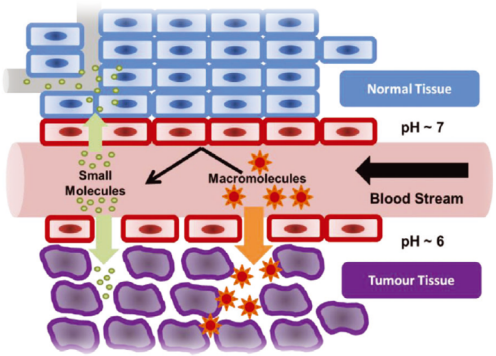


Figure 4. Enhanced permeability and retention effect. Adapted from: Blunden & Stenzel, 2015

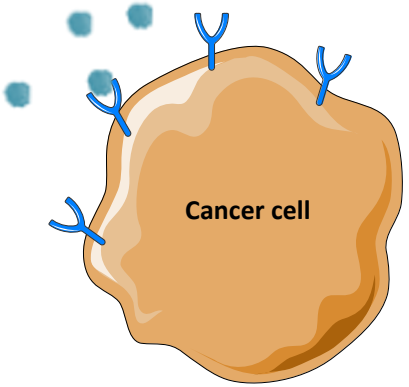


Figure 5. Biotin receptor (Sodium dependent multivitamin transporter) overexpression in cancer cells.

Legend:

Cytotoxic core **Biodegradable polymer chain** **Biotin molecule**

Aim

Study the anticancer effect of new Ru-cyclopentadienyl agents for colorectal cancer therapy

Two CRC-derived cell lines

SW480 (KRAS)

RKO (BRAF)

"Normal" colon-derived cell line

NCM460

Specific aims:

- What is the effect of ruthenium compounds in colorectal cancer cells survival?
- Which is the mechanism of action and molecular targets of these compounds?

Results and discussion

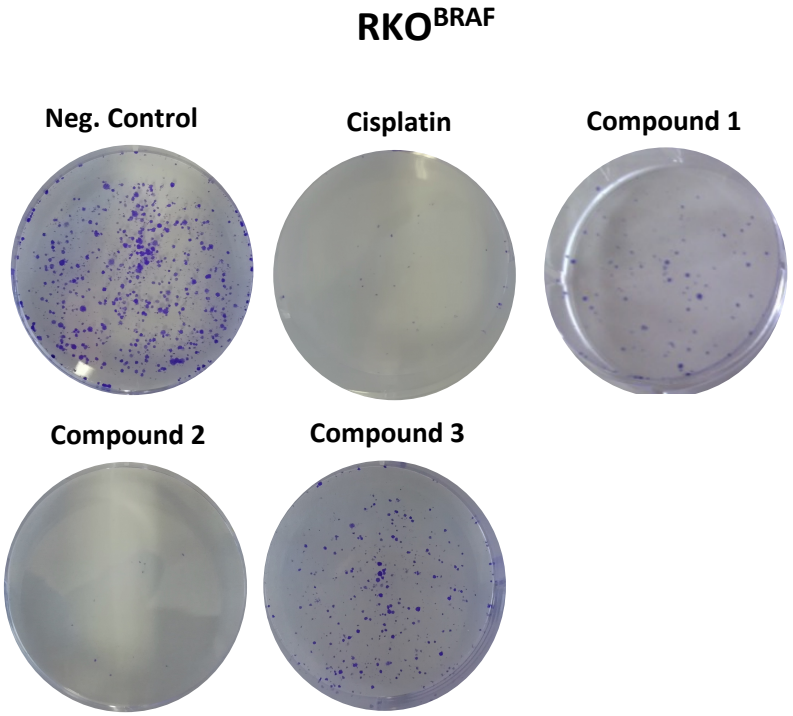
1. Ru agents are more cytotoxic and selective to CRC cells

Table 1. Determination of the IC_{50} values of Ru agents by Sulforhodamine B.

	$SW480^{KRAS}$	RKO^{BRAF}	NCM460	Selectivity Index	
Compound	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	SW480	RKO
1	6,0	4,0	15,0	2,5	3,8
2	14,1	7,7	57,2	4,1	7,4
3	1,8	2,8	4,3	2,4	1,5

Results and discussion

2. Ru agents decrease the colonogenic ability of CRC cells



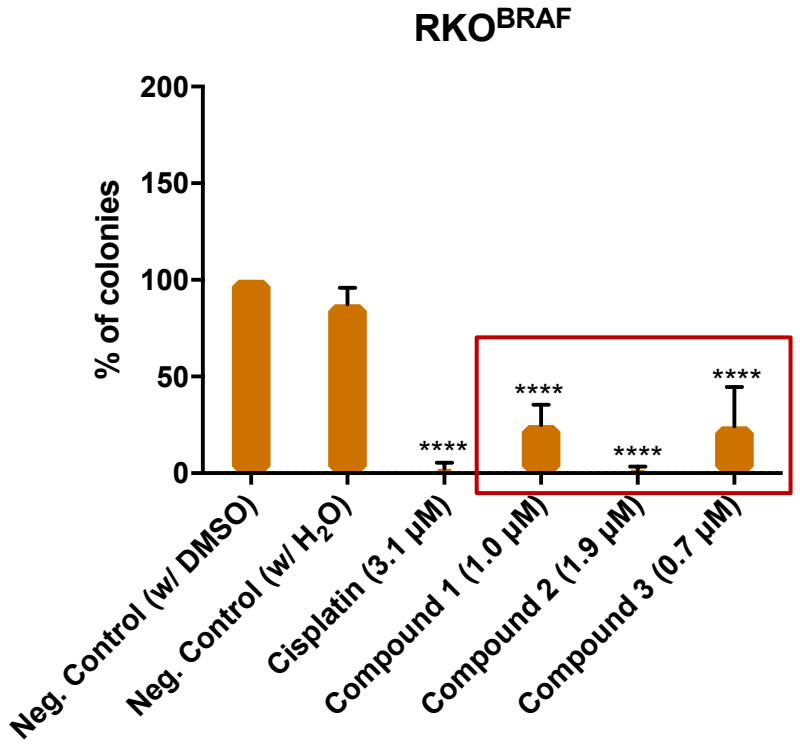


Figure 6. Representative images and graphics of colony formation ability of RKO cells.

Results and discussion

3. Ru agents decrease the proliferation of CRC cells

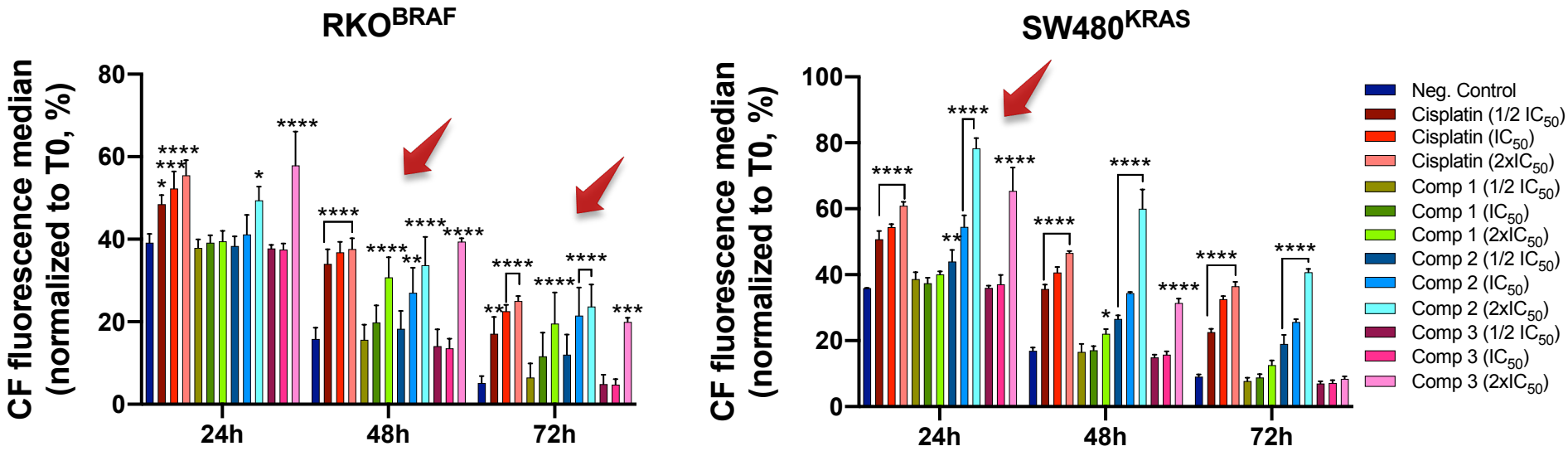


Figure 7. Graphical representation of proliferation analysis using carboxyfluorescein succinimidyl ester by flow cytometry.

Results and discussion

4. Ru agents induce cell cycle arrest in CRC cells

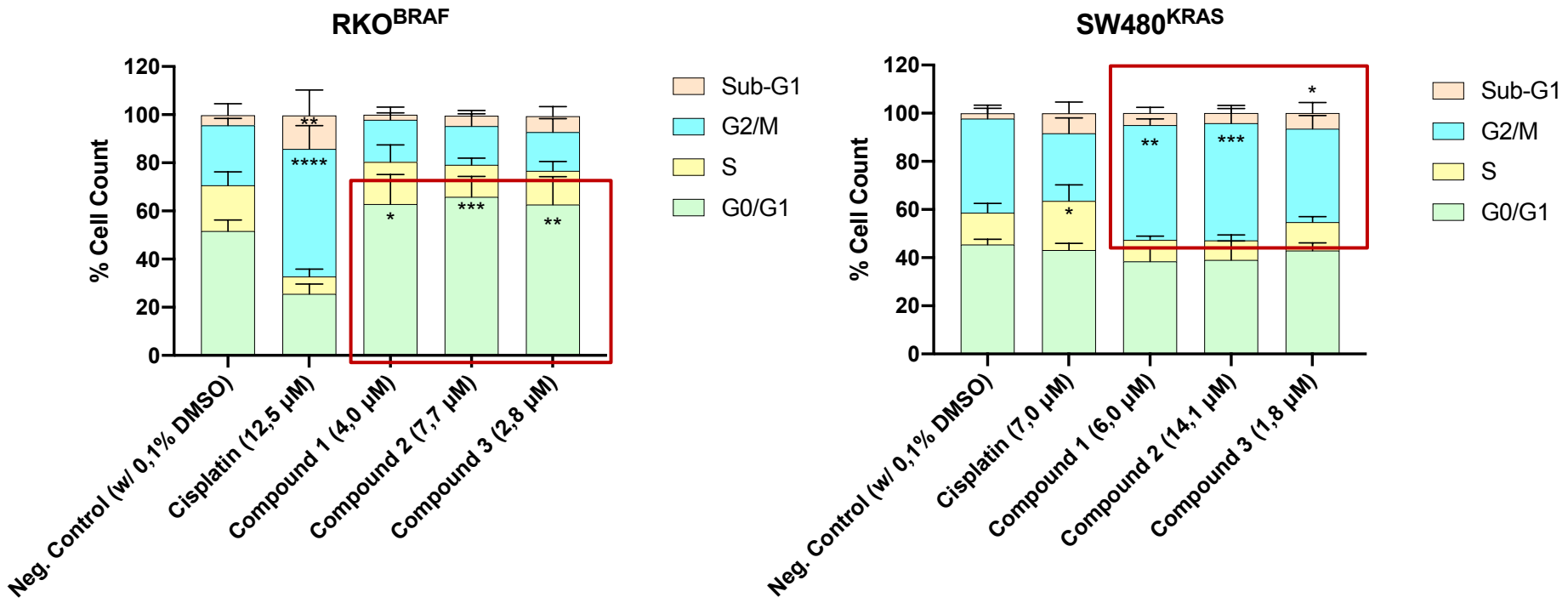


Figure 8. Graphical representation of cell cycle analysis using propidium iodide in CRC cells.

Results and discussion

5. Ru agents induce apoptosis in CRC cells

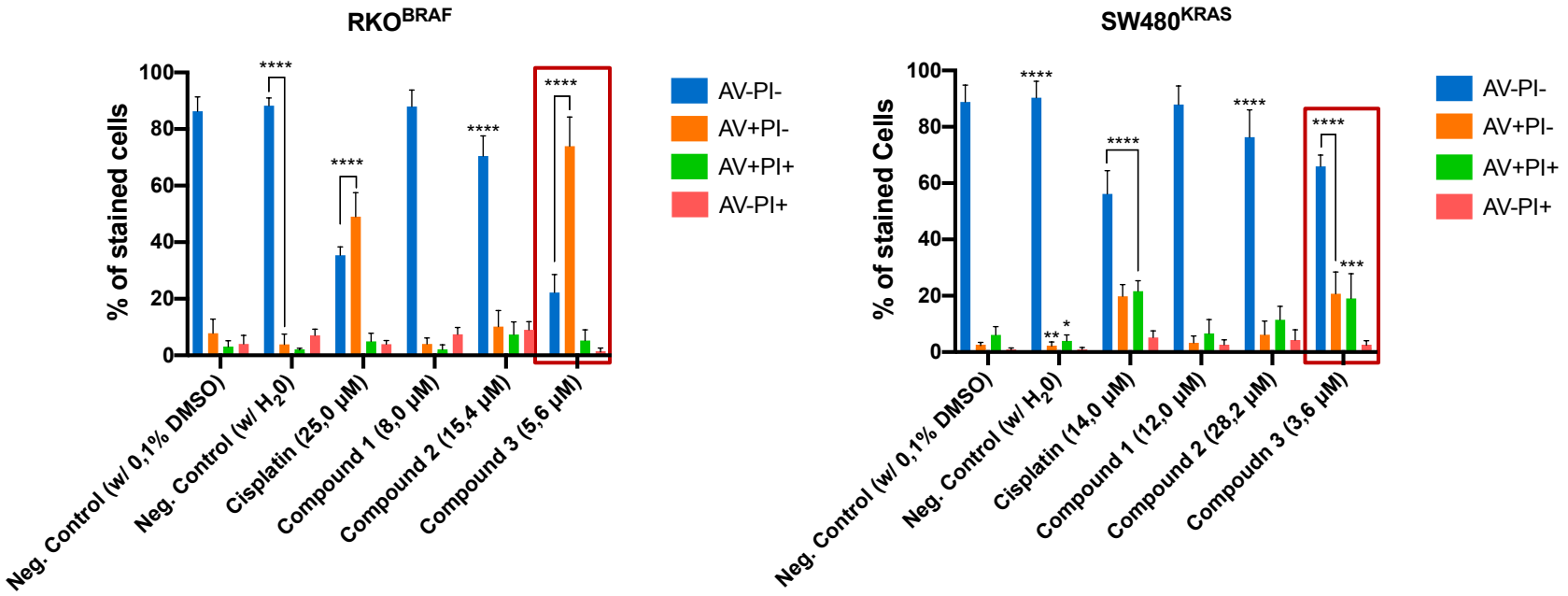


Figure 9. Graphical representation of annexin V/propidium iodide results in CRC cells.

Results and discussion

6. Ru agents are located at the cytoskeleton and membrane of CRC cells

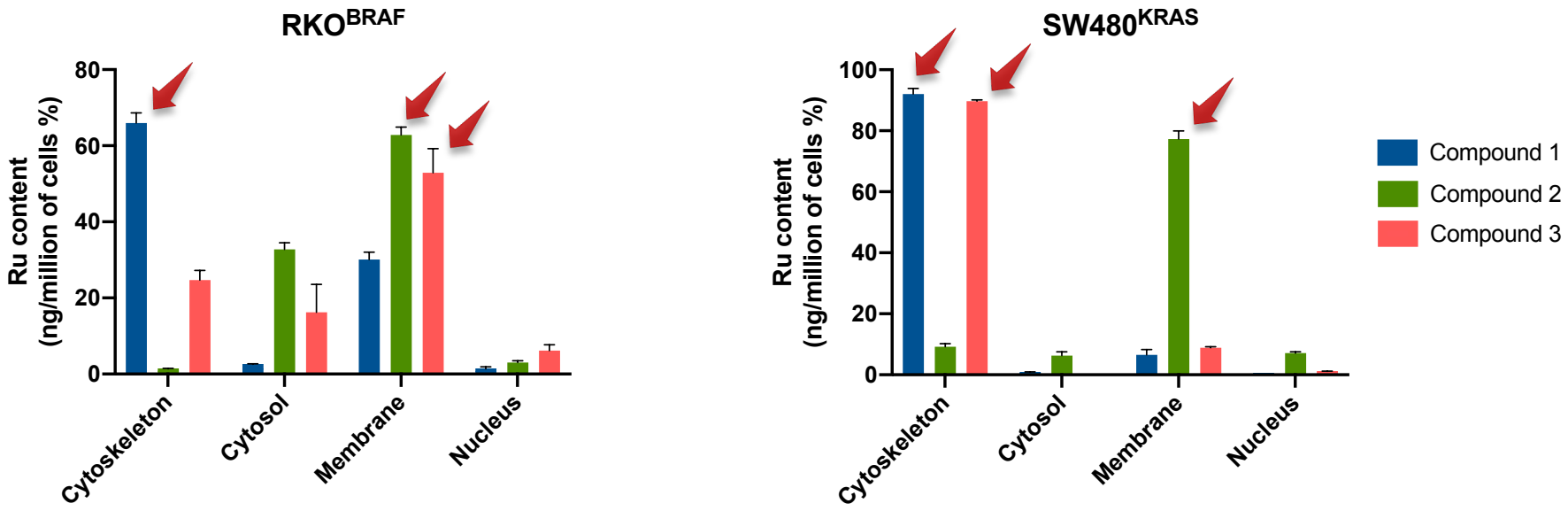


Figure 10. Graphical representation of cellular distribution of Ru agents in RKO and SW480 cells.

Results and discussion

7. Ru agents affect the actin cytoskeleton of CRC cells

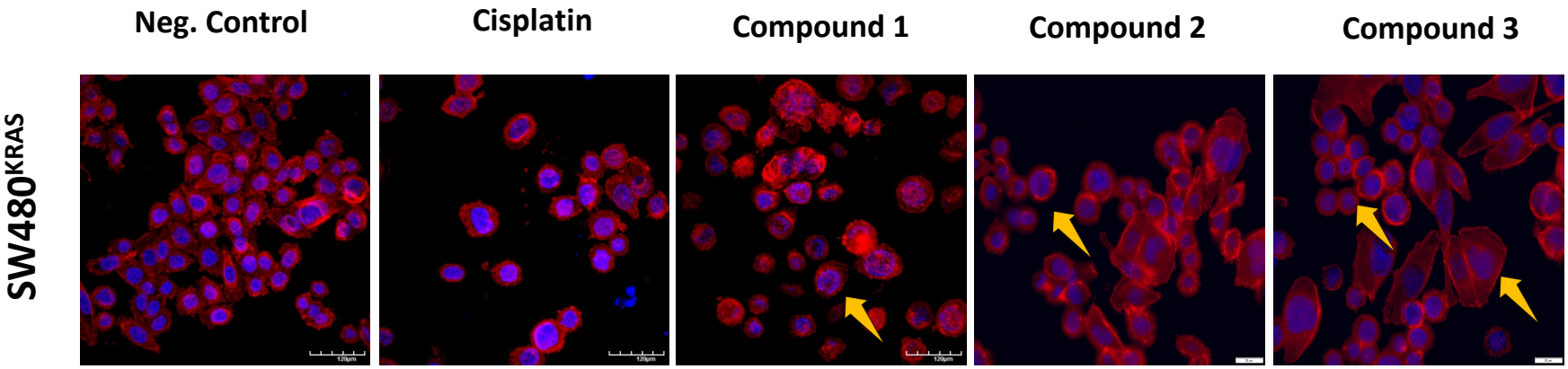


Figure 11. Analysis of F-actin staining in CRC cells. Representative images (×600) of DAPI, Phalloidin-AlexaFluor® 568 were obtained by confocal microscopy.

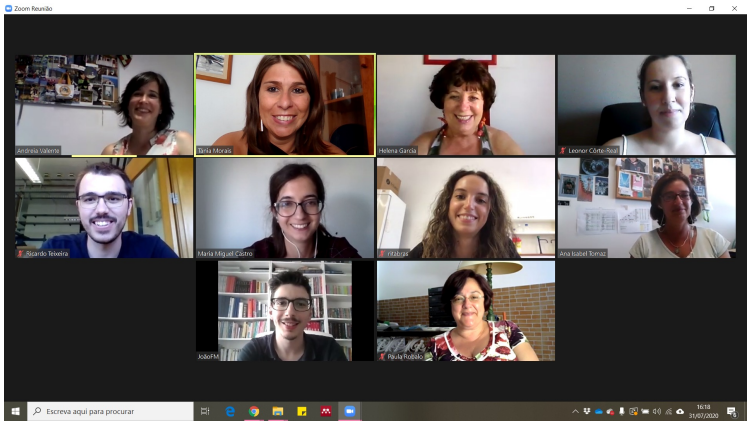
Conclusions

- ✓ Ru agents show **cytotoxicity** and **selectivity** towards CRC cells;
- ✓ Ru agents **decrease proliferation** and **induce apoptosis**;
- ✓ Ru agents **preferentially localize in membrane and cytoskeleton** in CRC cells;
- ✓ Ru agents also **affect F-actin polymerization.**

Ru agents showed promising anticancer activity in CRC cells.

Acknowledgments

Ana Preto's Group



Andreia Valente's Group

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