

Investigation of cardioprotective effect of PDE inhibitors in cardiomyocyte damaged by anthracyclines

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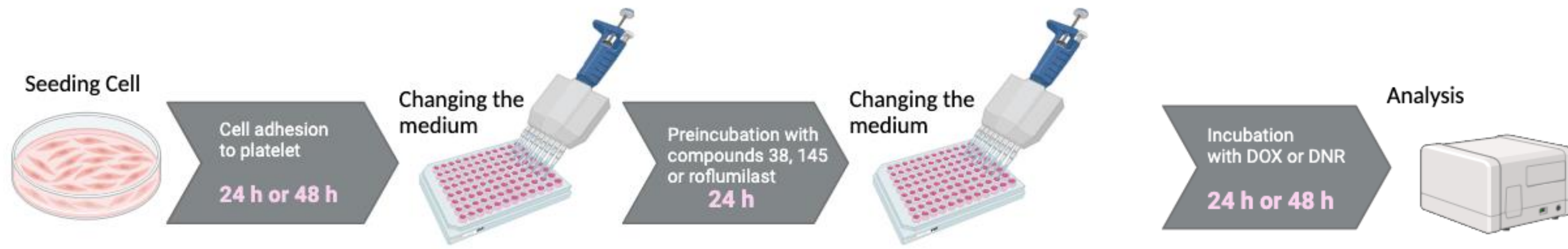
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Introduction

Anthracyclines antibiotics have been used for many years in the treatment of various types of cancer. They are characterized by high efficacy; however, therapy involving them is associated with numerous side effects, including cardiotoxicity. The mechanism of cardiotoxicity is multifactorial, related to the induction of oxidative stress, disturbances in calcium and iron ion homeostasis, and impairment of mitochondrial function. Dexrazoxane is the only drug registered as a cardioprotectant. Due to the involvement of cGMP and cAMP in regulating numerous pathological processes in the cardiac muscle, such as cardiomyoblast apoptosis and fibrosis of the heart muscle tissue. This study hypothesizes that PDE inhibitors may have a protective effect on cardiomyoblasts damaged by anthracyclines.

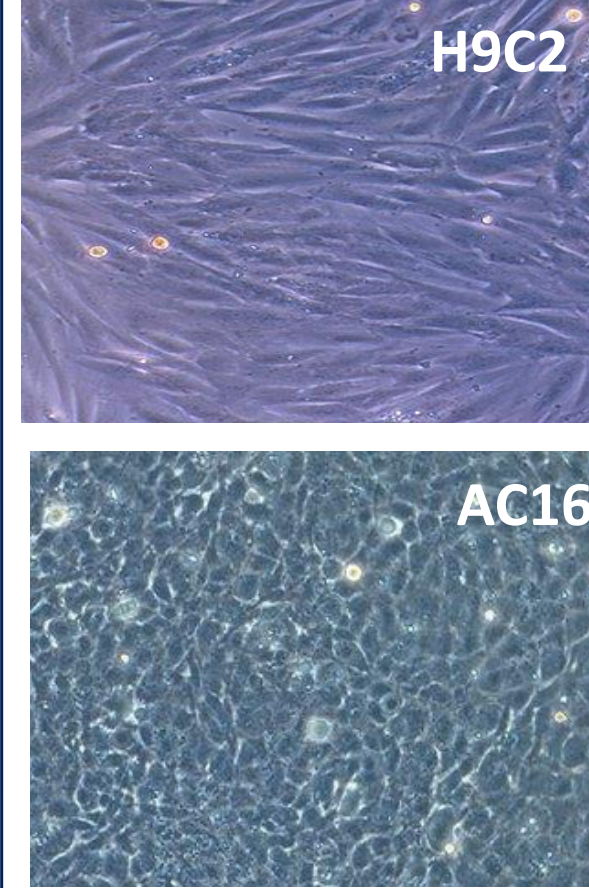
Experimental procedure



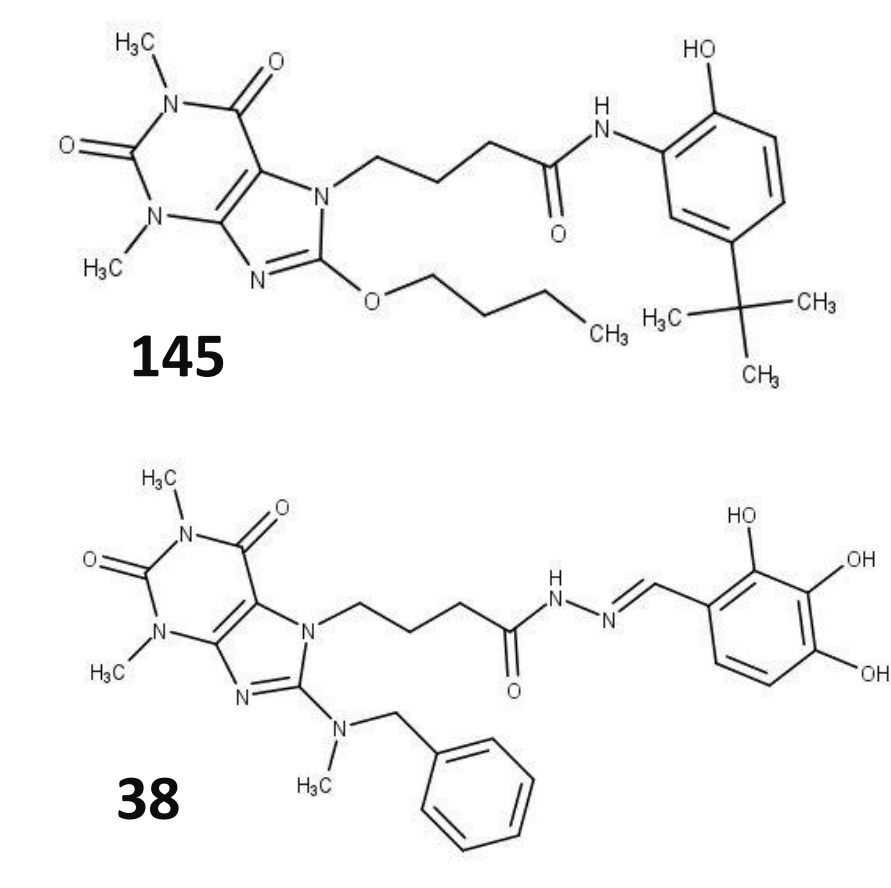
Aim of study

The aim of the study was to investigate the protective effects of new, non-selective PDE inhibitors (compounds 38 and 145) and a selective PDE4 inhibitor - roflumilast (RFL), on AC16 and H9C2 cardiomyocytes damaged by doxorubicin (DOX) and daunorubicin (DNR).

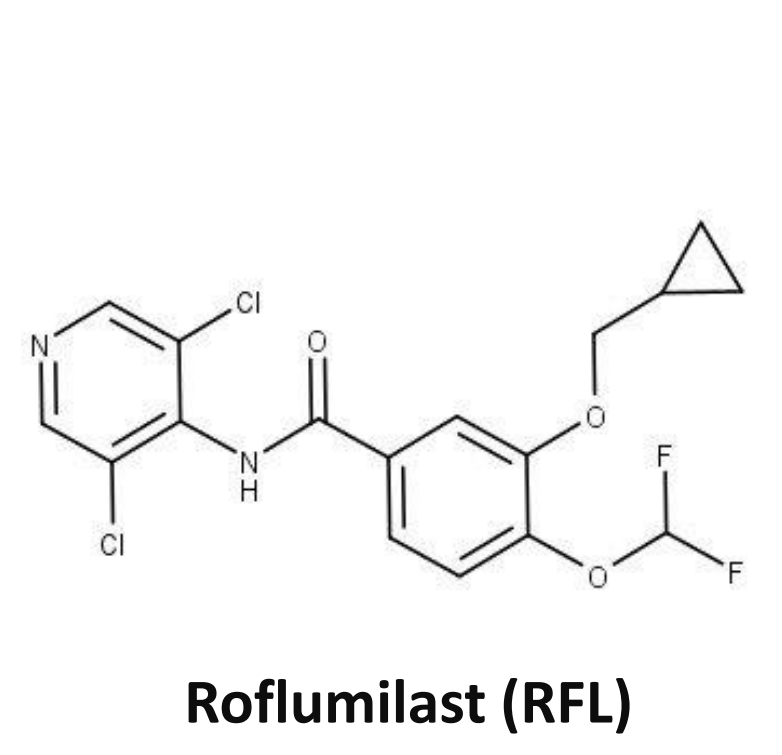
Cell lines



Tested compounds



Reference



Results

Viability – MTT assay

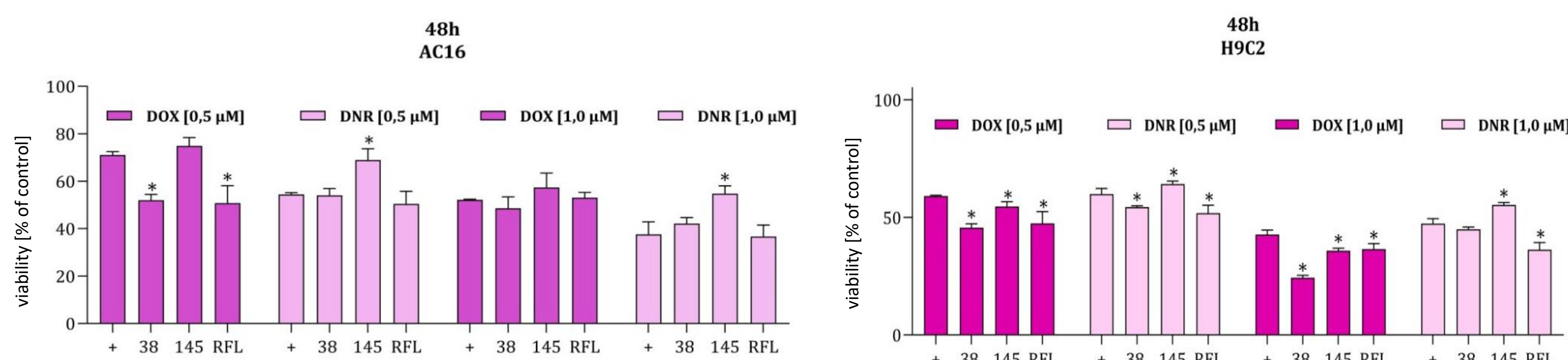


Fig.1. The effect of 38 or 145 on viability of human and rat cardiomyocytes treated with DOX or DNR. AC16 – human cardiomyocytes and H9c2 – rat cardiomyocytes were seeded into 96 well plates as density of 5000/well. Cells were preincubated in the presence of 38 or 145 alone for 24h, then DOX or DNR was added for next 48 hours. The graph represents the mean from three independent experiments expressed as percentage of the control \pm SD. $p < 0,05$ Mann Whitney U-test. Statistical analysis was performed using Graph Pad Prism Software.

Cells morphology

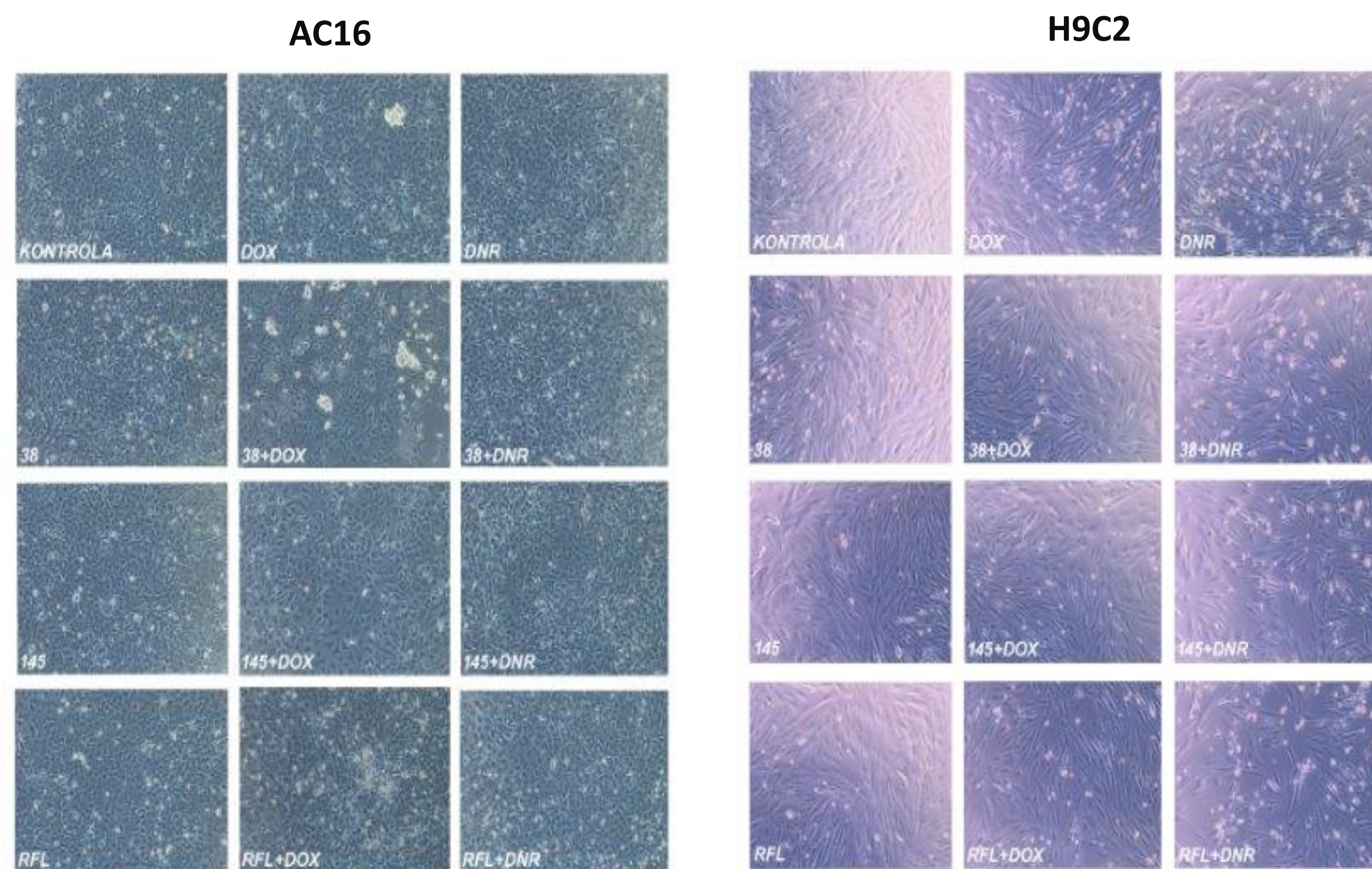
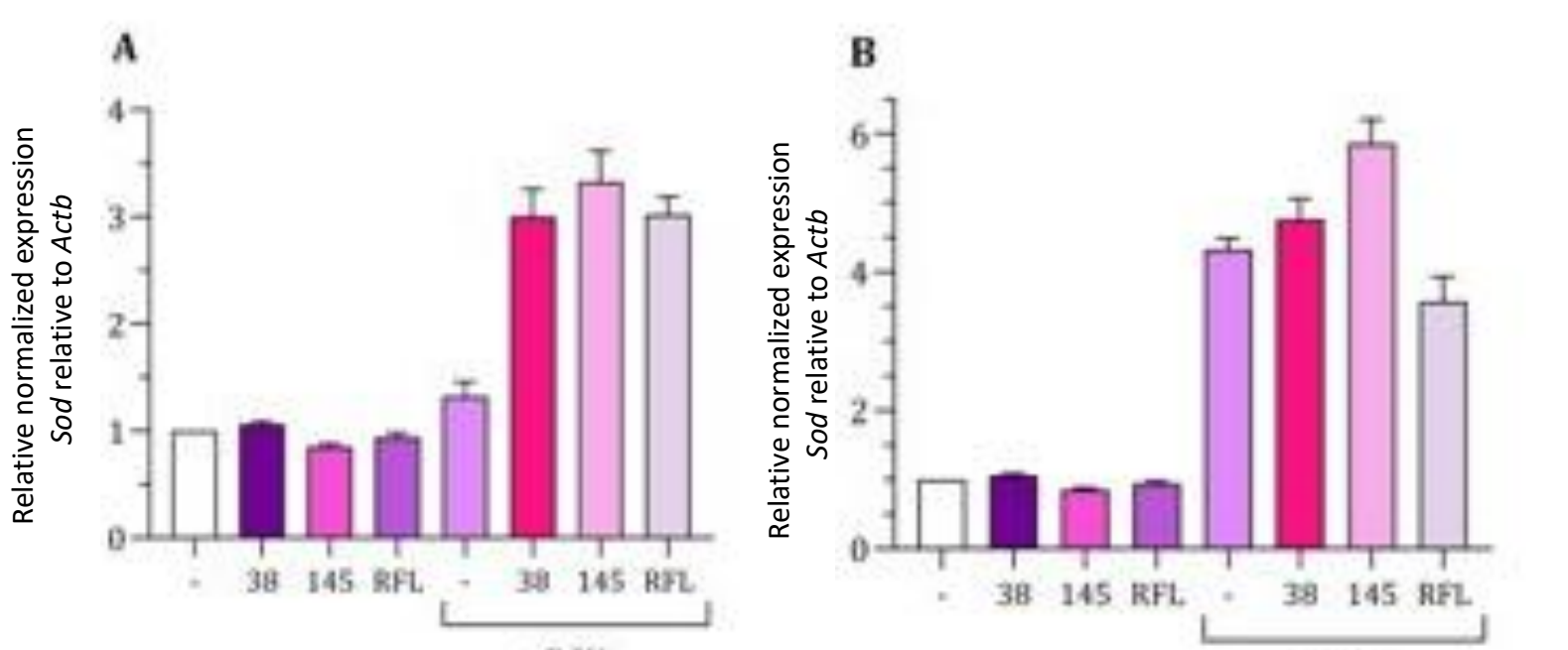


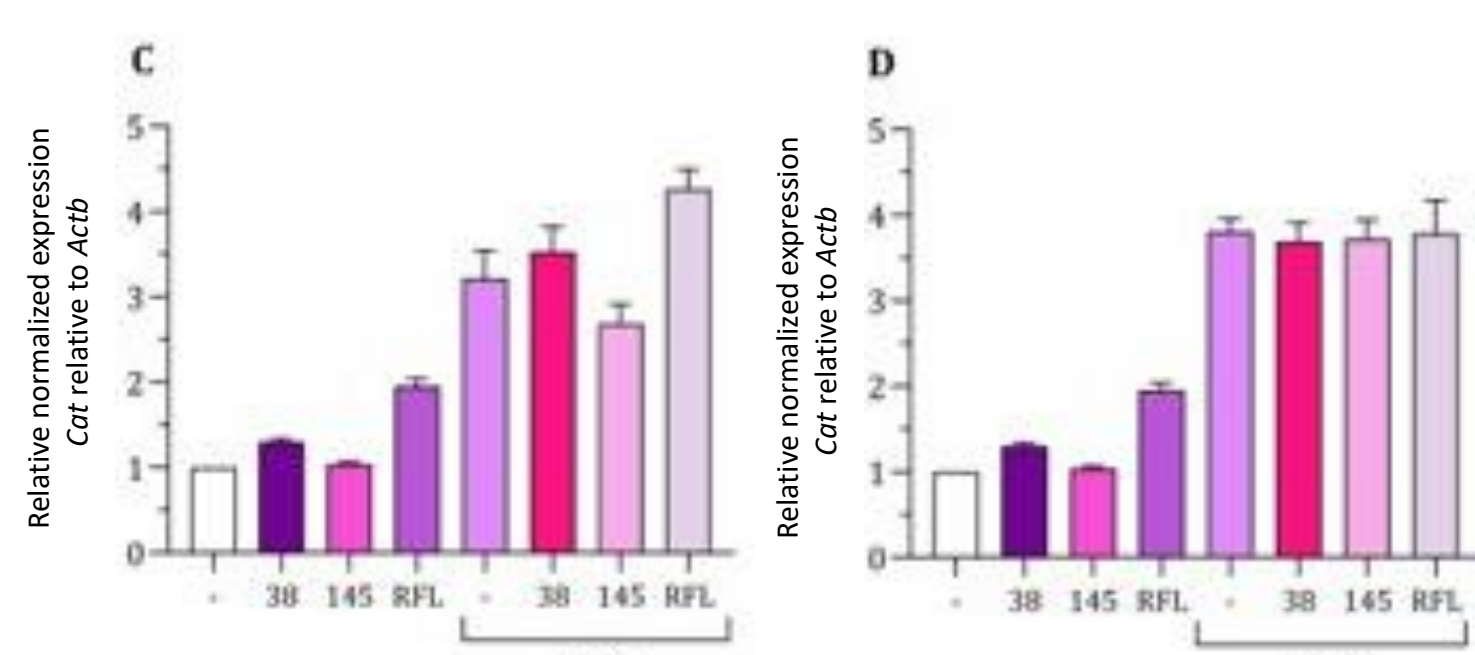
Fig.2. The effect of 38 or 145 on morphology of human and rat cardiomyocytes treated with DOX or DNR. Evaluation of cell morphology was performed on 24-well plates on which cells were seeded at a density of 30000 cells/well/0.5 mL of full culture medium. Compounds 38, 145 and RFL were used at a concentration of 10 μ M, while DOX and DNR were used at a concentration of 0.5 μ M. For morphology analysis, cells were observed under a Leica DMI 1 microscope with a camera and images were taken in the Leica Application Suite X PC program.

Gene expression analysis by qPCR

SOD gene - superoxide dismutase



Cat gene - catalase



Hmox1 gene - haem oxygenase

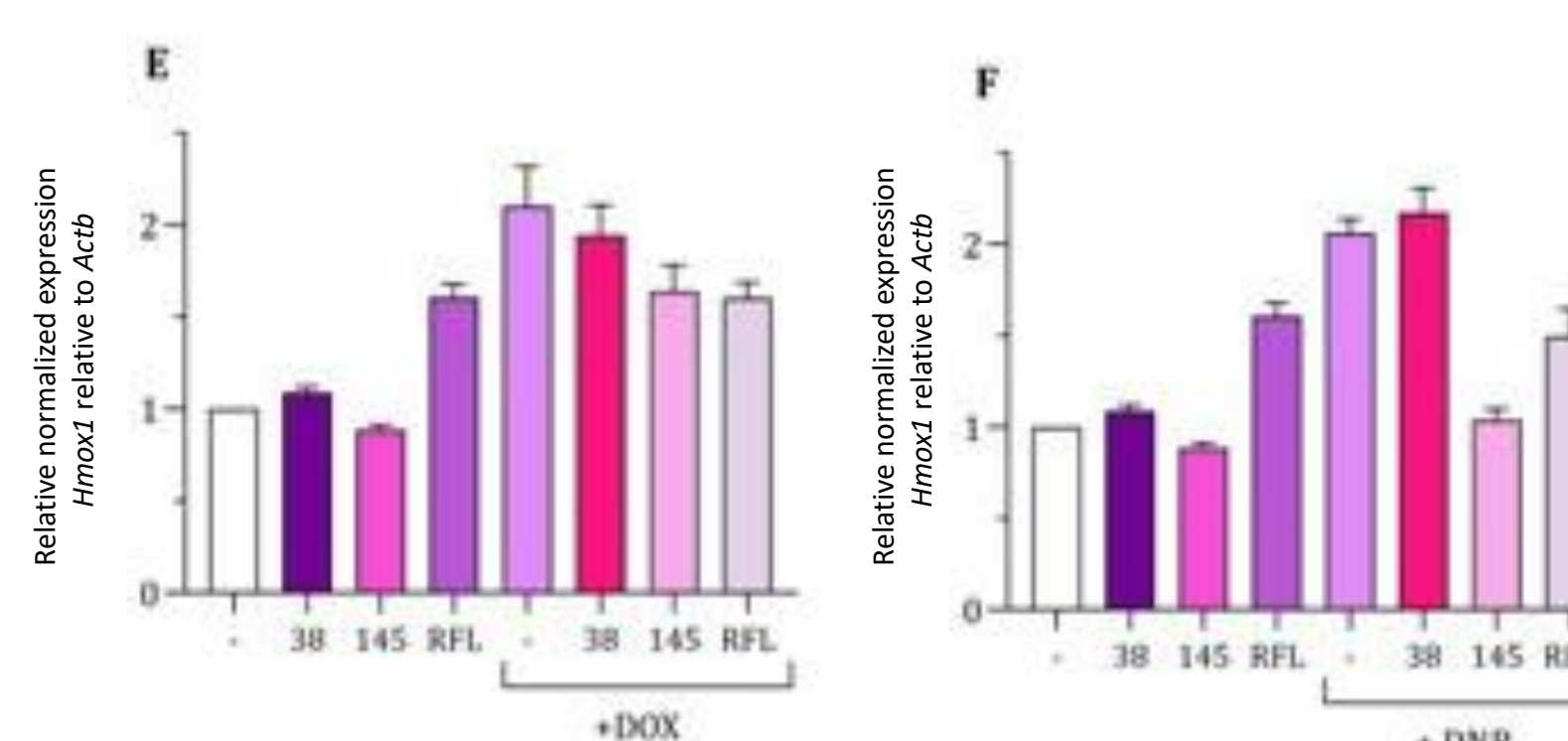
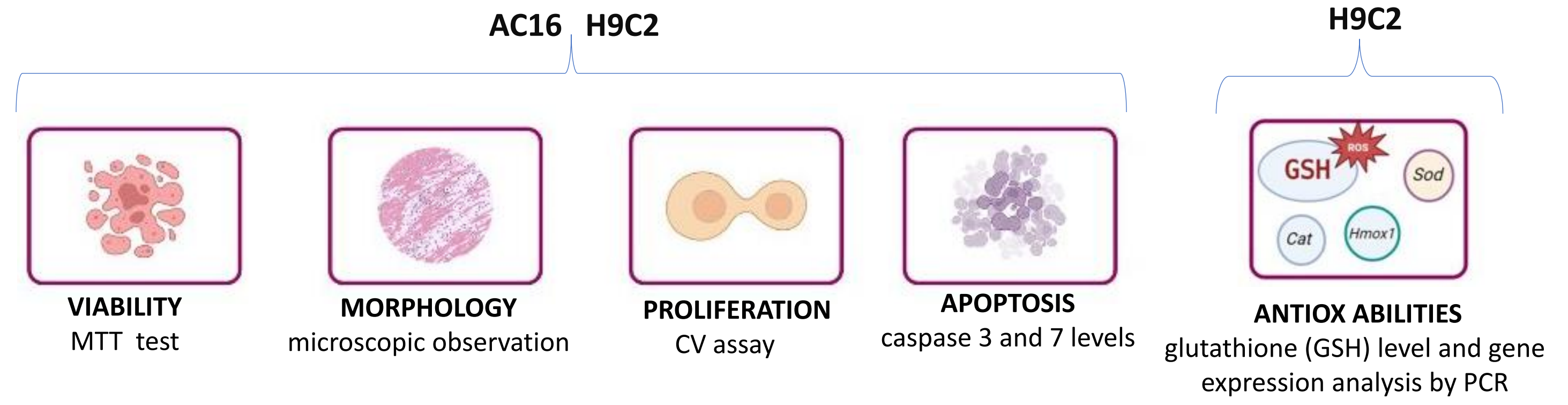


Fig.6. Effect of PDE inhibitors (38, 145, and RFL) on the expression of genes associated with the neutralization of oxidative stress in anthracycline-damaged H9C2 cardiomyocytes (DOX or DNR). The graphs show the effect on the expression of the following genes: Sodium (A, B), Cat (C, D), Hmox1 (E, F). Cells were incubated with compounds alone – 38, 145, RFL (10 μ M) or damaging agent alone (DOX and DNR), and cardiomyoblasts were pre-incubated with compounds 38 and 145 (10 μ M) or the reference substance RFL (10 μ M). The graphs represent the average fold of change in the expression of a analyzed gene relative to Actb \pm SD.

Methods



Caspase 3 and 7 level

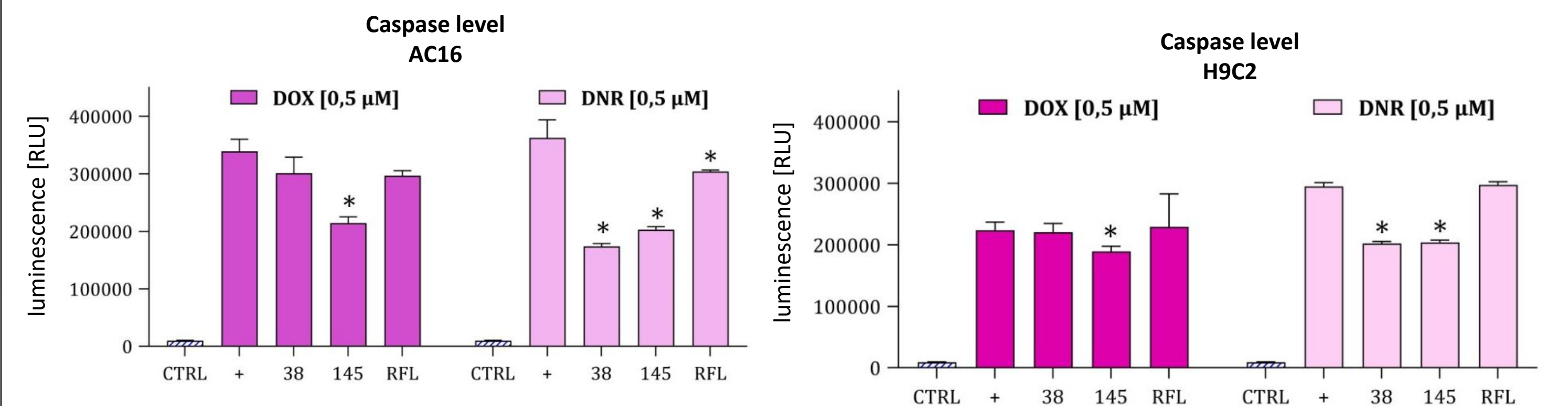


Fig.3. The effect of 38 or 145 on caspases 3 and 7 level of human and rat cardiomyocytes treated with DOX or DNR.

The level of caspases (3 and 7) – enzymes associated with the induction of apoptosis in AC16 and H9C2 cells preincubated with PDE inhibitors (38, 145, RFL) and damaged by anthracyclines (DOX or DNR). Cardiomyoblasts were pre-incubated with the study compounds 38 and 145 (10 μ M) or the reference substance RFL (10 μ M) for 24h, and then DOX or DNR (0.5 μ M) was damaged. The graph shows the average luminescence value [RLU] \pm SD. The control (CTRL) consisted of cells not with compounds. Statistical significance was calculated for cells incubated with DOX or DNR (+) only. Statistical significance (*), $p < 0,05$.

Proliferation

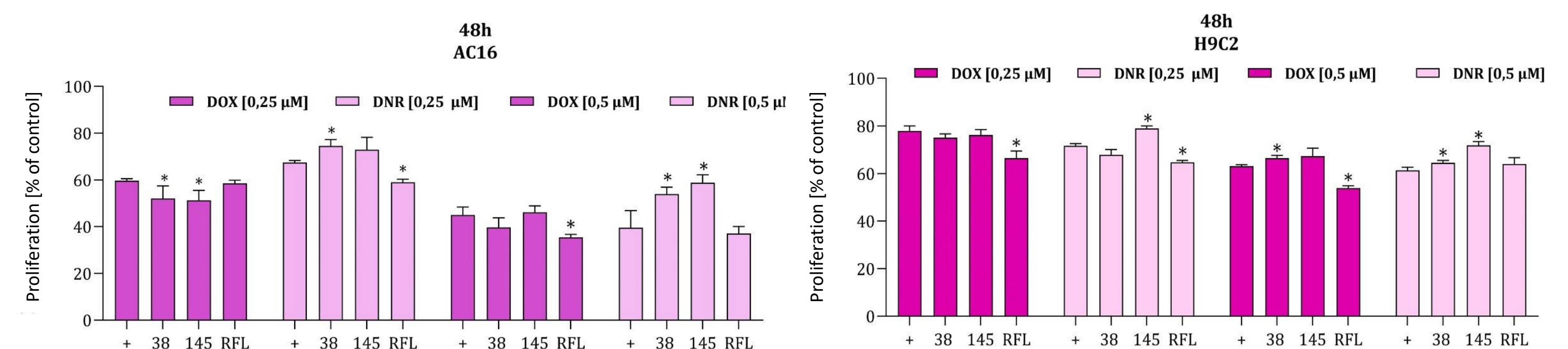


Fig.4. The effect of 38 or 145 on proliferation of human and rat cardiomyocytes treated with DOX or DNR. Cells were preincubated with PDE inhibitors (38, 145, and RFL) for 24 h and then were treated with DOX or DNR for next 48 h. Crystal violet assay was performed to analyzed cells proliferation. Graph represent average cell proliferation rate \pm SD. Statistical significance was calculated relative to cells incubated only with DOX or DNR (+). $p < 0,05$.

Glutathione (GSH) level

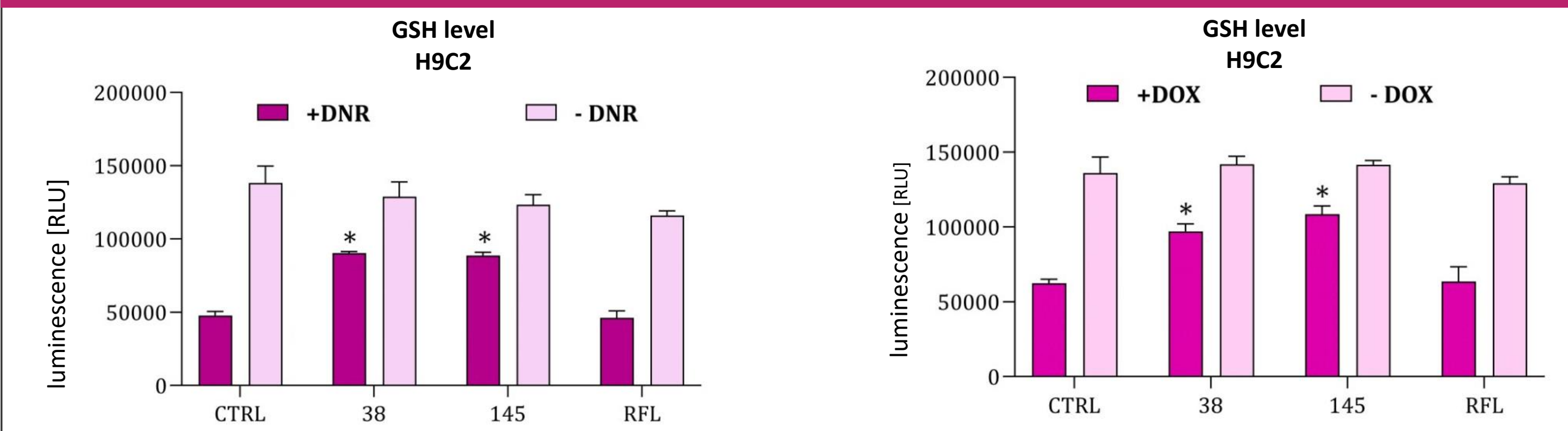


Fig.5. The effect of compounds 38 or 145 on GSH level on rat cardiomyocytes treated with DOX or DNR. Cells were preincubated with PDE inhibitors (38, 145, and RFL) for 24 h and then were treated with DOX or DNR for next 24 h. GSH glo assay was performed to evaluate the level of GSH in cells. The graph represent the average luminescence values [RLU] \pm SD. Statistical significance was calculated for cells incubated with DOX or DNR (+) only. Statistical significance (*), $p < 0,05$.

Summary and conclusions

- ✓ 145 reduced the cytotoxic effect of DNR on cardiomyocyte
- ✓ preincubation with 145 led to a reduction in caspase levels and oxidative stress in DOX and DNR-damaged cardiomyocytes
- ✓ 38 caused a decrease in caspase levels in DNR-damaged cells and prevented a decrease in glutathione levels in cardiomyocytes damaged by both DNR and DOX
- ✓ preincubation of cardiomyocytes with 38 increased DOX cytotoxicity in MTT test
- ✓ roflumilast has no cardioprotective effect

The obtained results may serve as a basis for further research into the use of 145 and 38 as potential cardioprotectants in anthracycline-induced heart damage; however, the safety of 38 should also be examined.

References



↑ GSH