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SUPPLEMENTARY MATERIALS

Article

Newly synthetized oxygenated xanthones as potential P-glycoprotein activators—*in vitro*, *ex vivo* and *in silico* studies

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Academic Editor: name
Received: date; Accepted: date; Published: date

Figure S1

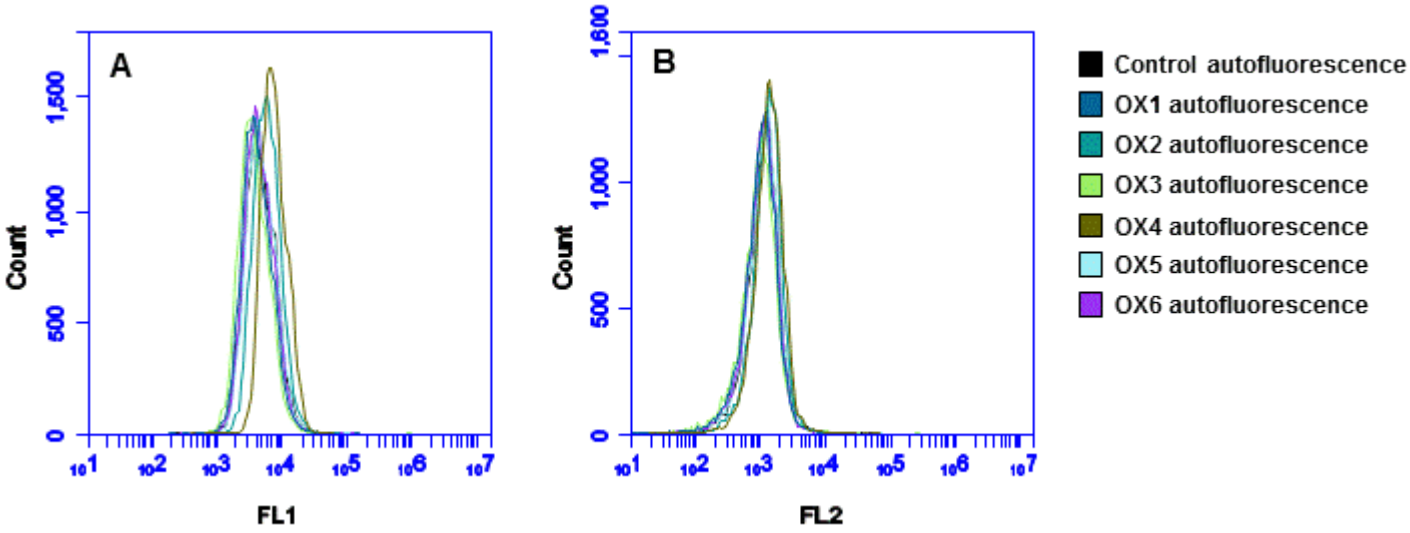

Figure S1. Representative histograms of flow cytometry analysis of Caco-2 cells autofluorescence in the 530 ± 15 nm band-pass filter (A - FL1 detector) and in the 585 ± 40 nm band-pass filter (B - FL2 detector), 24 h after the incubation with the tested oxygenated xanthones OX 1-6 (20.0 μM).

Figure S2

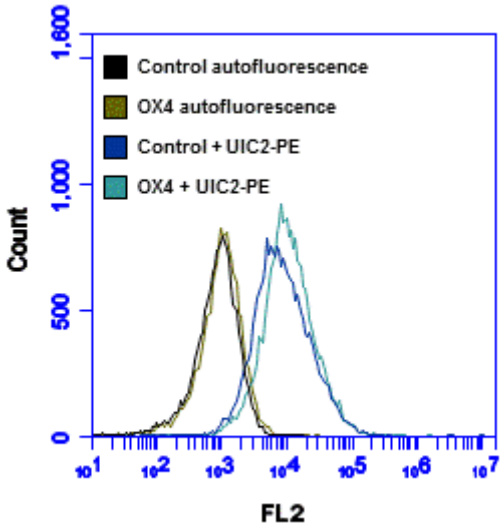

Figure S2. Representative histograms of flow cytometry analysis of P-glycoprotein (P-gp) expression, evaluated 24 h after exposure of Caco-2 cells to the oxygenated xanthone OX4 (20.0 μM), using the UIC2-PE monoclonal antibody (585 ± 40 nm band-pass filter - FL2 detector).

Figure S3

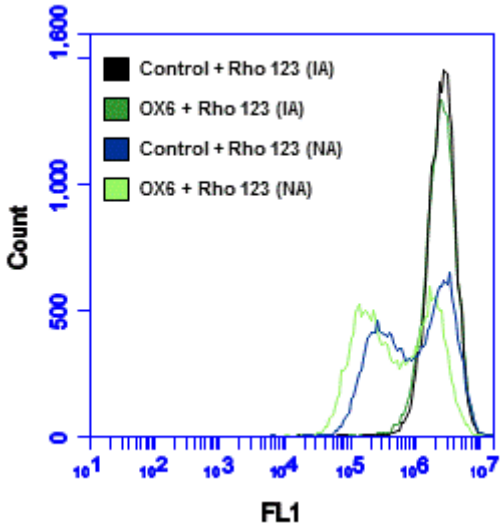

Figure S3. Representative histograms of flow cytometry analysis of P-glycoprotein (P-gp) activity, evaluated 1 h after exposure of Caco-2 cells to the oxygenated xanthone OX6 (20.0 μM), using Rhodamine (Rho 123) as a fluorescent substrate (530 ± 15 nm band-pass filter - FL1 detector). IA (inhibited accumulation), NA (normal accumulation).

Figure S4

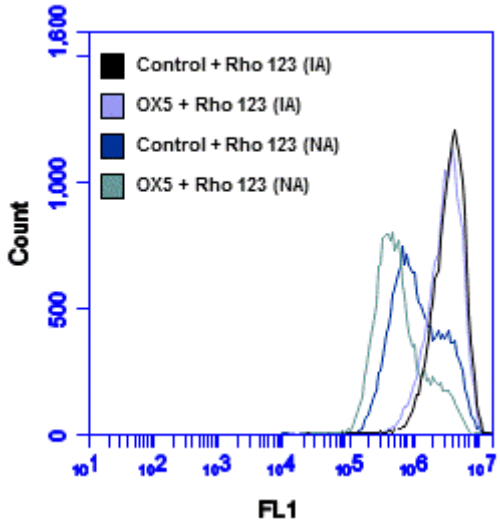

Figure S4. Representative histograms of flow cytometry analysis of P-glycoprotein (P-gp) activity, evaluated 24 h after exposure of Caco-2 cells to the oxygenated xanthone OX5 (20.0 μM), using Rhodamine (Rho 123) as a fluorescent substrate (530 ± 15 nm band-pass filter - FL1 detector). IA (inhibited accumulation), NA (normal accumulation).

Figure S5

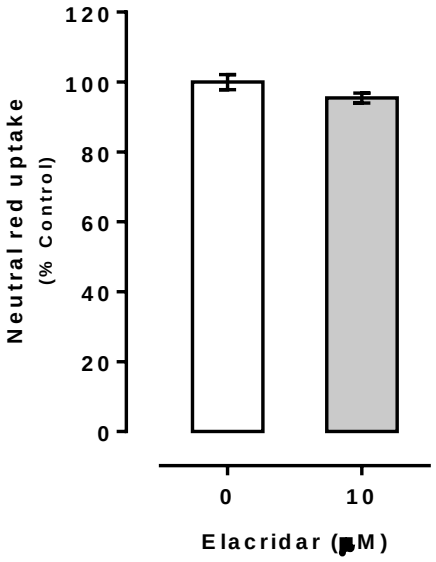

Figure S5. Elacridar (Ela, 0 - 10.0 μM) cytotoxicity in Caco-2 cells evaluated by the Neutral Red (NR) uptake assay, 24 h after incubation. Results are presented as mean ± SEM from 3 independent experiments, performed in triplicate. Statistical comparisons were made using the Unpaired *t* test.

Figure S6

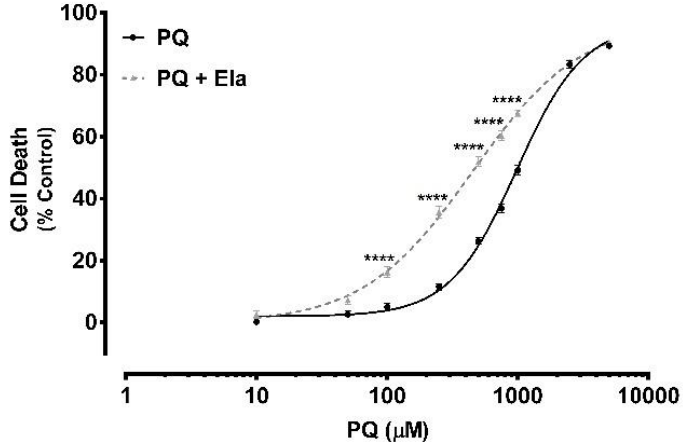

Figure S6. Paraquat (PQ) concentration-response (cell death) curve obtained in the absence (PQ) or in the presence of 10.0 μM Elacridar (PQ + Ela). Results are presented as mean ± SEM from 4 independent experiments (performed in triplicate). Concentration-response curve was fitted using least squares as the fitting method and the comparison between PQ and PQ + Ela curves (LOG EC_{50}, TOP, BOTTOM, and Hill Slope) was made using the extra sum-of-squares F test. Statistical comparisons were made using Two-way ANOVA, followed by the Sidak's multiple comparisons post hoc test (****$p < 0.0001$ PQ + Ela vs. PQ). In all cases, p values < 0.05 were considered statistically significant.

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Table S1

Table S1. EC_{50} (half-maximum-effect concentrations), TOP (maximal effect), BOTTOM (baseline) and Hill Slope values of the paraquat (PQ) concentration-response curve, with (PQ + Ela) or without (PQ) simultaneous exposure to Elacridar (10.0 μM).

	PQ	**PQ + Ela**
EC_{50} (half-maximum-effect concentrations, μM)	**982.4**	**450.3******
Top (maximal cell death, % control)	96.65	97.76
Bottom (baseline, % control)	2.008	-0.2621
Hill slope	**1.694**	**1.039******
Curve *p* value (Comparison between the fitted curves)	-	**< 0.0001**

Concentration-response curves were fitted using least squares as the fitting method and the comparisons between PQ and PQ + Ela curves were made using extra sum-of-squares F test. In all cases, p values < 0.05 were considered significant (****p < 0.0001 for PQ vs. PQ + Ela). Bold is used when significant exists.