Supporting information

Iron Chelators and Exogenic Photosensitizers. Synergy through Oxidative Stress Gene Expression

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Experimental section

Synthesis

Thiosemicarbazones (TSCs) and **chlorin c** were synthesized as described previously [1–3].

Pharmacological synergy

The cells were seeded in 3 cm Petri dishes (Nunc), ($2 \cdot 10^5$ cells/dish) 24h before the experiment. The medium was removed the next day and freshly prepared solutions of TSCs and PSs were added. After 24h cells were washed with PBS (Immuniq) and 1 mL of DMEM

(without phenol red and without FBS) were added. Then cells were irradiated with a light of 660 nm in the dose of 12 J/cm^2. Then medium was replaced with a standard medium and after the next 24h, the viability of the cells was determined using the MTS test; 130 µL of the CellTiter 96® AQueous One Solution – MTS (Promega) solution was added to each dish (with 700 µL DMEM without phenol red) and incubated for 15min at 37°C. The optical densities of the samples were analyzed at 490 nm. The experiments were repeated at least three times. The calculation of the synergy was performed according to the Chou-Talay method (Compusyn software) [4–6]. Briefly, the concentrations scope was adjusted to cover the specified IC_{50} value for each drug. Four concentrations of TSCs and PSs were tested. The doses were increased at a constant ratio according to refs [4–6] (**Table S1**).

The combination index CI was calculated according to equation

$$\frac{(D)_{TSC}}{(D_x)_{TSC}} + \frac{(D)_{PDT}}{(D_x)_{PDT}} = CI$$

where D_x is the dose of PDT or TSC given alone, enough to reach effect x (x% of inhibition). D is the portion of PDT or TSC given in combination sufficient to achieve the effect x.

Fraction affected f_a was calculated according to equation

$$f_a = \left(\frac{D}{D_m}\right)^m / f_u$$

where D is the dose of the TSC or PDT, D_m jest the dose of the TSC or PDT causes 50% effect, m describes the shape of the dose-response curve, f_u is unaffected fraction.

Table S1. F_a fractions resulted from the respective TSCs and PSs doses.

	chlorin c				
MS168	dose [µM]	0,5	0,75	1,125	1,6875
dose [nM]	F_a	0,016833	0,418129	0,639269	0,834125
100	0,429606	0,678231			
150	0,489585		0,746698		
225	0,591237			0,886666	
337,5	0,625996				0,949374
	Foscan				

MS168	dose [µM]	0,3	0,45	0,675	1,0125
dose [nM]	F_a	0,173234	0,46591	0,811082	0,833075
100	0,429606	0,785964			
150	0,489585		0,835715		
225	0,591237			0,929583	
337,5	0,625996				0,948547

	chlorin c				
Dp44mT	dose [µM]	0,5	0,75	1,125	1,6875
dose [nM]	F_a	0,016833	0,418129	0,639269	0,834125
60	0,729576	0,893037			
90	0,764709		0,916646		
135	0,829712			0,947499	
202,5	0,835901				0,979946
	Foscan				
Dp44mT	dose [µM]	0,3	0,45	0,675	1,0125
dose [nM]	F_a	0,173234	0,46591	0,811082	0,833075
60	0,729576	0,907667			
90	0,764709		0,965182		
135	0,829712			0,981574	
202,5	0,835901				0,971275

	chlorin c				
3-AP	dose [µM]	0,5	0,75	1,125	1,6875
dose [µM]	F_a	0,016833	0,418129	0,639269	0,834125
25	0,423814	0,633134			
37,5	0,414174		0,901683		
56,25	0,450812			0,929922	
84,375	0,411336				0,970113
	Foscan				
3-AP	dose [µM]	0,3	0,45	0,675	1,0125
dose [µM]	F_a	0,173234	0,46591	0,811082	0,833075
25	0,423814	0,836592			
37,5	0,414174		0,926413		
56,25	0,450812			0,958094	
84,375	0,411336				0,949547

	chlorin c				
DFO	dose [µM]	0,5	0,75	1,125	1,6875
dose [µM]	F_a	0,016833	0,418129	0,639269	0,834125
10	0,066948	0,355803			
15	0,092945		0,4756		
22,5	0,105631			0,716203	
33,75	0,057495				0,901149
	Foscan				
DFO	dose [µM]	0,3	0,45	0,675	1,0125

dose [μM]	F_a	0,173234	0,46591	0,811082	0,833075
10	0,066948	0,123841			
15	0,092945		0,48424		
22,5	0,105631			0,755506	
33,75	0,057495				0,810382

Oxidative stress mRNA expression of Mn-SOD and CAT

Table S2. Primer pairs sequences used in determination of mRNA expression of MnSOD, CAT and GADPH.

Gene	GenBank accession no.	Forward primer (5′→3′)	Reverse primer (5′→3′)
MnSOD	NM_001024465.1	AAACCTCAGCCCTAACGGTG	CCAGGCTTGATGCACATCTTA
CAT	NM_001752.3	ACTGTTGCTGGAGAATCGGG	AAGTCTCGCCGCATCTTCAA
GADPH	NM_002046	GAGTCAACGGATTTGGTCGTA	GCCCCACTTGATTTTGGAG

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