SUPPORTING INFORMATION

Design and Evaluation of Multi-functional Nanocarriers for Selective Delivery of Coenzyme Q10 to Mitochondria

Anjali Sharma[a,‡], Ghareb M. Soliman[a,b,c,‡], Noura Al-Hajaj[b], Rishi Sharma[a], Dusica Maysinger[*,b], Ashok Kakkar[**,a]

[a]Department of Chemistry, McGill University, 801 Sherbrooke St. West, Montreal, Quebec, H3A 2K6, Canada

[b]Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir-William-Osler, Montreal, Quebec, H3G 1Y6, Canada

[c]Permanent Address: Department of Pharmaceutics, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

[‡] These authors contributed equally

*Corresponding author. Tel.: + 514-398-1264; Fax: +514-398-6690

**Corresponding author. Tel. +514-398-6912; Fax: +514-398-3797.

E-mail addresses: dusica.maysinger@mcgill.ca (D. Maysinger)*, ashok.kakkar@mcgill.ca (A.Kakkar)**

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1. **Synthesis of a series of targeted and non-targeted PEG-PCL miktoarm polymers:**

PEG2-PCL3.5-Br: A solution of compound **6** (50mg, 0.02mmoles) in dry toluene (2 ml) was placed in a flame-dried two neck round bottom flask fitted with a condenser. The solution was degassed by evacuation, and distilled ε-caprolactone (0.07ml, 0.67mmoles) was added under nitrogen with a syringe through the rubber septum. A nitrogen purged solution of Sn(II) 2-ethylhexanoate (catalytic) in toluene (1 ml) was then added to the reaction flask, and the mixture was refluxed for 24 h. The reaction mixture was then cooled to room temperature, and the solvent was removed under vacuum. The product was dissolved in dichloromethane and precipitated in cold methanol. The polymer was filtered and washed with diethylether to yield a white powder. GPC: *Mn* = 5992, Polydispersity Index (PDI) =1.2.

PEG2-PCL3.8-Br: Compound **6** (50mg, 0.02mmoles) and ε-caprolactone (0.08ml, 0.74mmoles) were reacted together using the above mentioned procedure for ring opening polymerization. GPC: *Mn* = 6252, PDI =1.3.

PEG2-PCL4.4-Br: Compound **6** (50mg, 0.02mmoles) and ε-caprolactone (0.10ml, 0.88mmoles) were reacted together using the above mentioned procedure for ring opening polymerization. GPC: *Mn* = 6911, PDI =1.3.

PEG2-PCL5.5-Br: Compound **6** (50mg, 0.02mmoles) and ε-caprolactone (0.12ml, 1.10mmoles) were reacted together using the above mentioned procedure for ring opening polymerization. GPC: *Mn* = 7932, PDI =1.4.

PEG2-PCL7.0-Br: Compound **6** (50mg, 0.02mmoles) and ε-caprolactone (0.15ml, 1.37mmoles) were reacted together using the above mentioned procedure for ring opening polymerization. GPC: *Mn* = 9574, PDI =1.3.

PEG2-PCL7.8-Br: Compound **6** (50mg, 0.02mmoles) and ε-caprolactone (0.18ml, 1.58mmoles) were reacted together using the above mentioned procedure for ring opening polymerization. GPC: *Mn* = 10247, PDI =1.2.

PEG2-PCL3.5-TPP$^+$Br$^-$: A solution of PEG2K_PCL3500_Br (100mg, 0.02mmoles) and triphenyl phosphine (TPP) (8.8mg, 0.03mmoles) in acetonitrile (ACN) was refluxed for 48 h. The solvent was then evaporated and the residue was washed several times with hexanes and diethylether to remove excess of TPP. The white solid was then dried under vacuum. GPC: *Mn* = 6022, PDI =1.3.

PEG2-PCL3.8-TPP$^+$Br$^-$: PEG2K_PCL3800_Br (100mg, 0.02mmoles) and TPP (8.4mg, 0.03mmoles) were reacted together using a similar procedure as mentioned above to afford the product as white solid. GPC: *Mn* = 6284, PDI =1.4.

PEG2-PCL4.4-TPP$^+$Br$^-$: PEG2K_PCL4400_Br (100mg, 0.01mmoles) and TPP (7.6mg, 0.03mmoles) were reacted together using a similar procedure as mentioned above to afford the product as white solid. GPC: *Mn* = 7353, PDI =1.3.

PEG2-PCL5.5-TPP$^+$Br$^-$: PEG2K_PCL5500_Br (100mg, 0.02mmoles) and TPP (6.6mg, 0.03mmoles) were reacted together using a similar procedure as mentioned above to afford the product as white solid. GPC: *Mn* = 8374, PDI =1.4.

PEG2-PCL7.0-TPP$^+$Br$^-$: PEG2K_PCL7000_Br (100mg, 0.01mmoles) and TPP (5.5mg, 0.02mmoles) were reacted together using a similar procedure as mentioned above to afford the product as white solid. GPC: *Mn* = 11140, PDI =1.6.

PEG2-PCL7.8-TPP$^+$Br$^-$: PEG2K_PCL7800_Br (100mg, 0.01mmoles) and TPP (5.1mg, 0.02mmoles) were reacted together using a similar procedure as mentioned above to afford the product as white solid. GPC: *Mn* = 12072, PDI =1.6.

2. Supplementary Tables and Figures

Table 2.1. Calculated solubility parameters and Flory-Huggins interaction parameters for PCL, nimodipine and coenzyme Q10

Drug/Polymer	Total solubility parameter $(J/cm^3)^{1/2}$ [a]	χ_{sm} [b]
PCL	20.59	-
Nimodipine	26.10	4.57
Coenzyme Q10	18.14	2.54

[a]: Solubility parameters estimated by the Hansen theory of solubility group contribution method (GCM) using Molecular Modeling Pro software

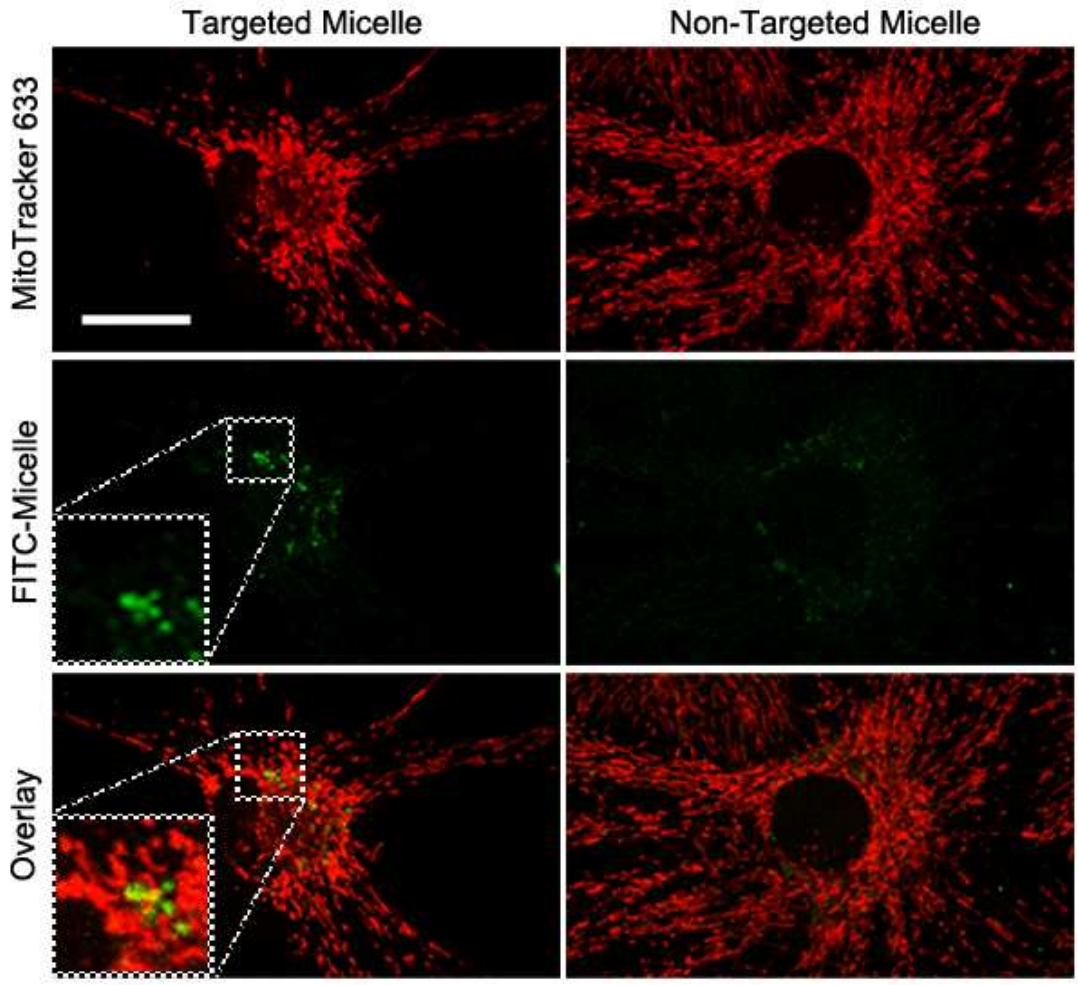
[b]: Flory-Huggins interaction parameters between PCL and different drugs calculated using equation 1

$$\chi_{sm} = \frac{(\delta_s - \delta_m)^2 V_s}{RT} \qquad (1)$$

δ_s and δ_m are solubility parameters for the drug and the micellar core, respectively; V_s is the molar volume of drug, R is the universal gas constant, and T is the Kelvin temperature.

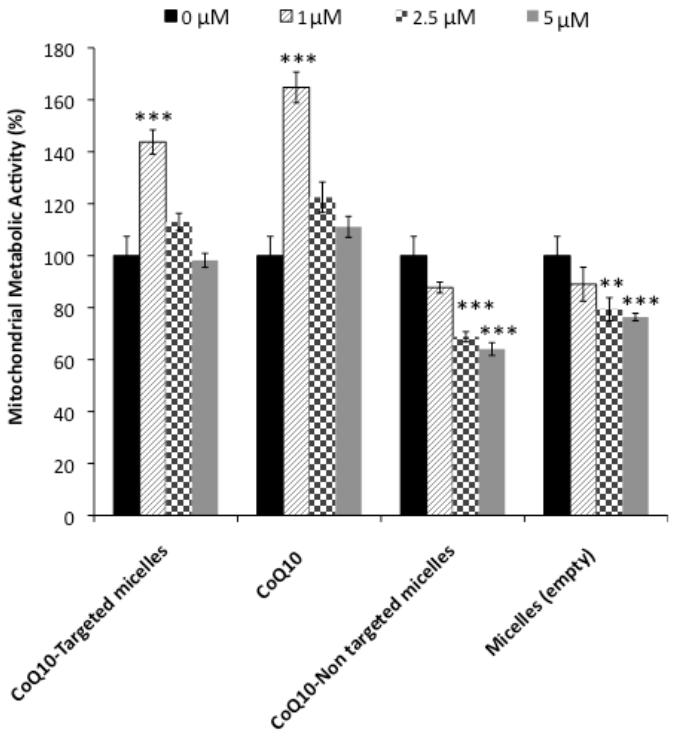
Table 2.2. Zero and first order kinetics for the degradation of CoQ10 under UV irradiation

Sample	R^2[a]		K[b]	$T_{0.5}$[c]
	Zero order	**First order**	(min^{-1})	(min)
CoQ10 solution (0.50 mg/mL)	0.976	0.966	0.10	6.85
CoQ10 micelles (0.10 mg/mL)	0.971	0.991	0.06	11.66
CoQ10 micelles (0.25 mg/mL)	0.966	0.998	0.04	19.05
CoQ10 micelles (0.50 mg/mL)	0.958	0.996	0.03	25.74
CoQ10 micelles (0.75 mg/mL)	0.981	0.998	0.01	56.67

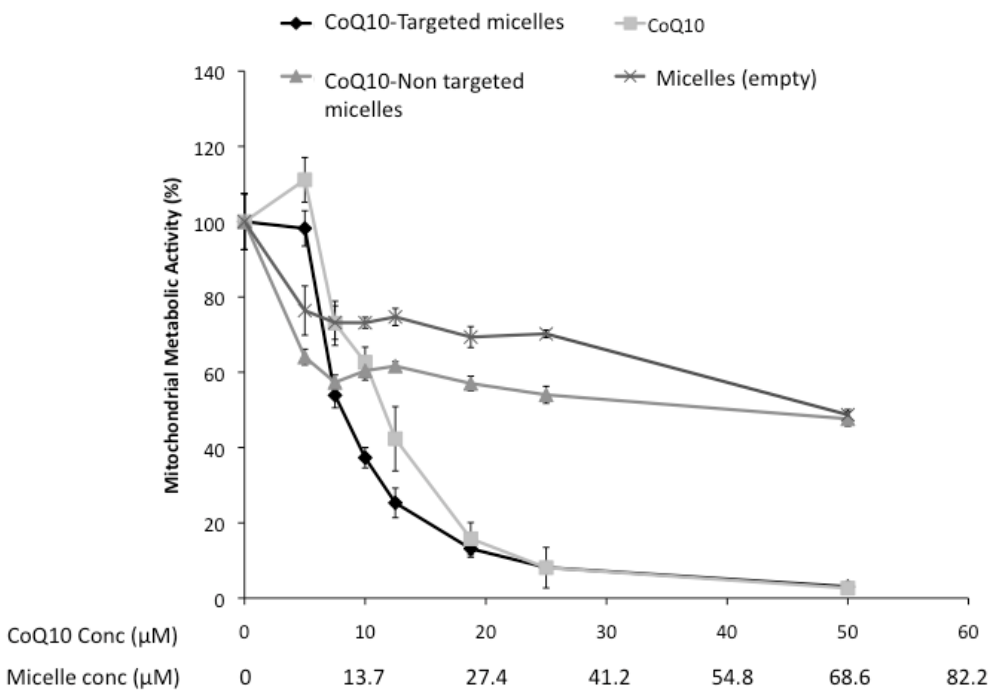


Supplemental Figure 3.1. FITC-Targeted micelle partially co-localize with mitochondria. Confocal micrographs of primary hippocampal neurons and glia cells treated with FITC-PEG2-PCL3.8-TPPBr (Targeted Micelle, 1 μM, 3 h, green fluorescence) or FITC-PEG2-PCL3.8-Br (Non-Targeted Micelle, 1 μM, 3 h, green fluorescence) and Mitotracker 633 (100 nM, 3 min, deep red fluorescence). FITC-Targeted micelle partially co-localize with mitochondria as shown in overlay image (inset, yellow), which was not found in cell cultures treated with FITC-Non targeted micelles (bottom, right panel). Each image represents a Z-stack consisting of 6-8 confocal sections taken at an interval of 0.3 μm. Images were acquired using HeNe (633 nm) and Argon (488 nm) excitation lasers for detection of deep red and green fluorescence, respectively. Scale bar (20 μm) is representative for all images.

A)

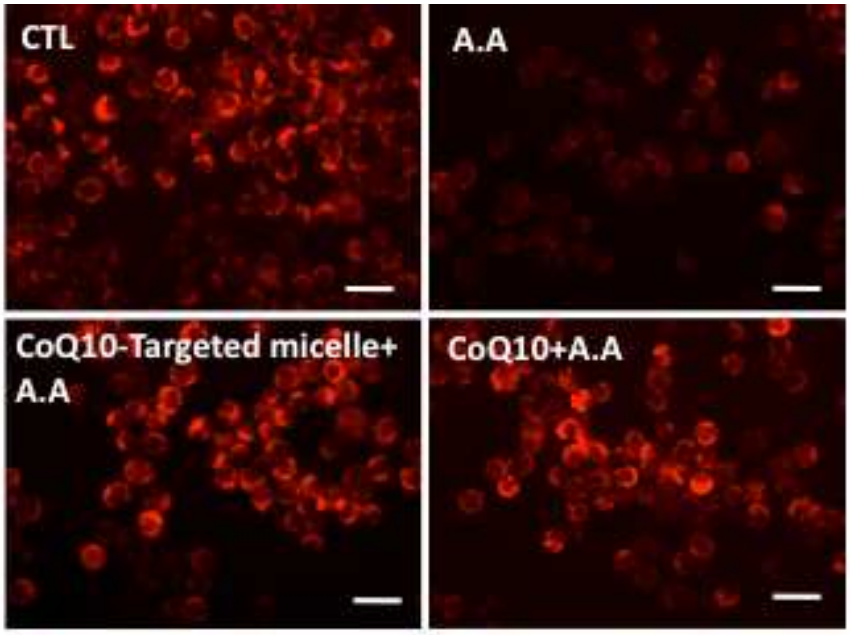

0 µM
1 µM
2.5 µM
5 µM
Mitochondrial Metabolic Activity (%)
180
160
140
120
100
80
60
40
20
0
***
***
***
***
**
***
CoQ10-Targeted micelles
CoQ10
CoQ10-Non targeted micelles
Micelles (empty)

B)

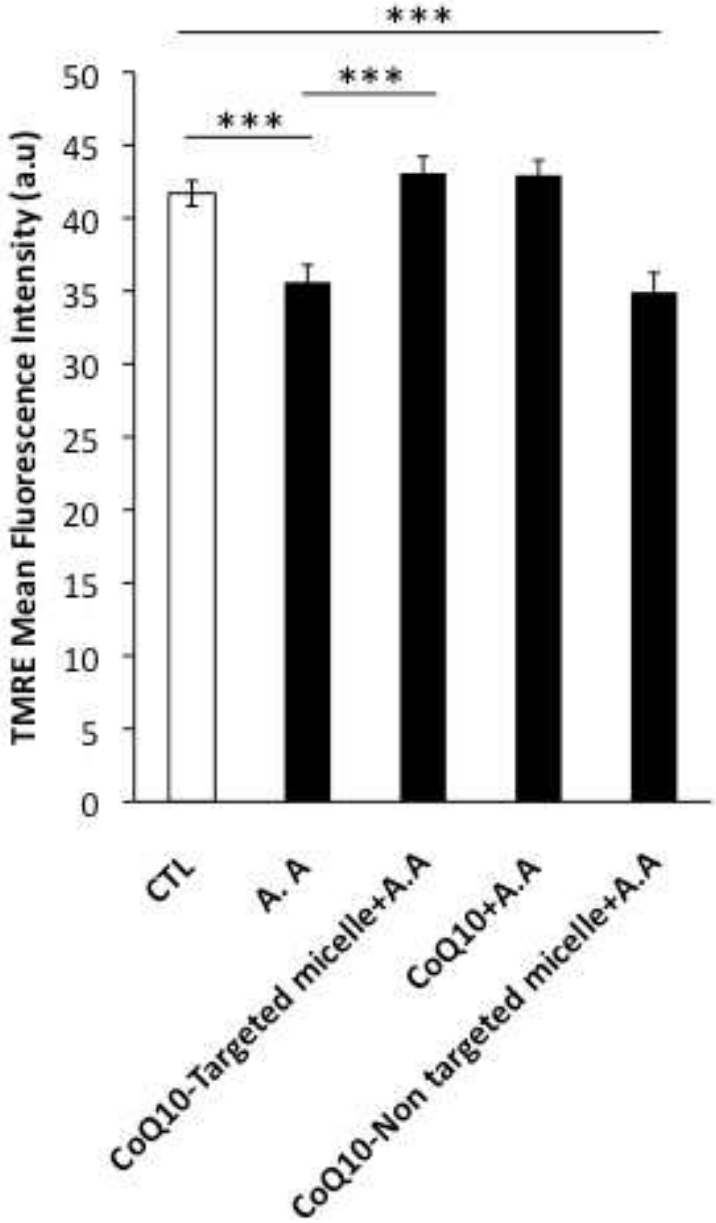

CoQ10-Targeted micelles
CoQ10
CoQ10-Non targeted micelles
Micelles (empty)
Mitochondrial Metabolic Activity (%)
140
120
100
80
60
40
20
0
CoQ10 Conc (µM)
0
10
20
30
40
50
60
Micelle conc (µM)
0
13.7
27.4
41.2
54.8
68.6
82.2

Supplemental Figure 3.2. Mitochondrial metabolic activity measured by MTT assay. Microglia cells treated with CoQ10, CoQ10/PEG2-PCL3.8-TPPBr (CoQ10-Targeted micelles), CoQ10/PEG2-PCL3.8-Br (CoQ10-Non targeted micelles) and PEG2-PCL3.8-TPPBr (empty micelles for 24 h (panels A and B). Mitochondrial metabolic activity (%) is expressed relative to controls (untreated cells that were set to 100%, n=9). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p** <0.01, p*** <0.001.

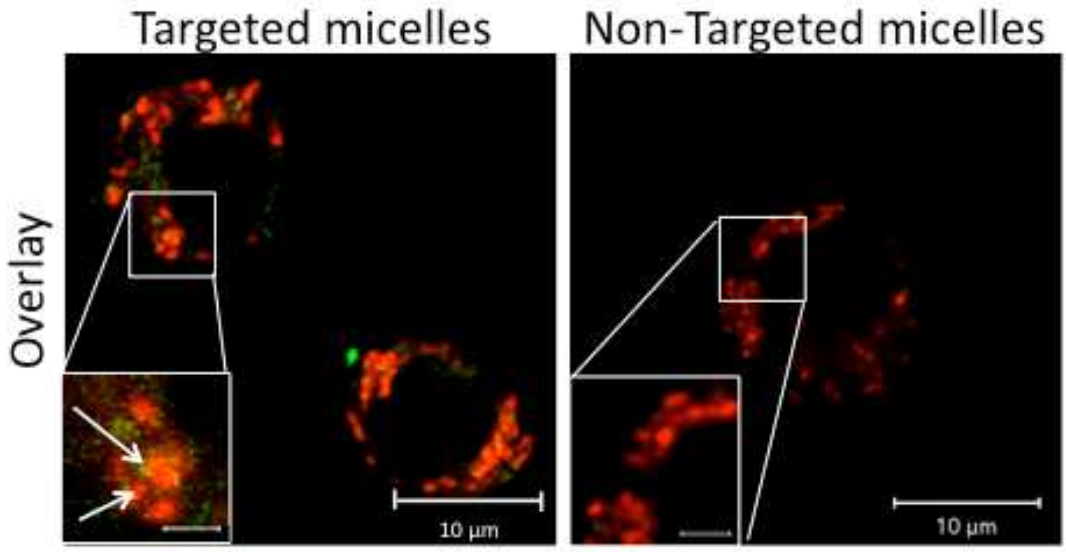
A)


CTL
A.A
CoQ10-Targeted micelle+ A.A
CoQ10+A.A

B)


***
***
***
TMRE Mean Fluorescence Intensity (a.u)
50
45
40
35
30
25
20
15
10
5
0
CTL
A. A
CoQ10-Targeted micelle+A.A
CoQ10+A.A
CoQ10-Non targeted micelle+A.A

Supplemental Figure 3.3. Microglia cells were treated with CoQ10/PEG2-PCL3.8-TPPBr (CoQ10-Targeted micelle), CoQ10, or CoQ10/PEG2-PCL3.8-Br (CoQ10-Non targeted micelle) (5 μM with respect to CoQ10, 24 h). (A) Fluorescent micrographs showing mitochondrial membrane potential using TMRE (200 nM, 30 min) following antimycin A exposure (A.A, 1 μM, 24 h). Scale bar =20 μm. (B) Semiquantification of TMRE mean fluorescence intensity (arbitrary units) was performed using Image J software from cells treated as in A (n=16). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p*** <0.001.



Supplemental Figure 3.4. Targeted micelles with non covalently bound FITC (green) partially co-localize with mitochondria (red). Confocal micrographs of microglia cells treated with targeted Micelle or Non-Targeted Micelles is shown. Each image represents a Z-stack consisting of 12 confocal sections taken at an interval of 0.6 μm. Scale bar (10 μm).

4. Detailed caption for Fig. 6 from the Manuscript. Production of reactive oxygen species (ROS). (A) Fluorescent micrographs showing reactive oxygen species generation following H_2O_2 exposure (2 mM, 3 h) using DCFH-DA, a fluorescent probe sensitive to ROS radicals. Scale bar= 20 μm. (B)

Spectrofluorometric detection and quantification of DCF fluorescence intensity (arbitrary units) was expressed relative to untreated controls (CTL=1, white bar) (n=8). (C) Fluorescent micrographs showing superoxide anion (O_2-) generation following PQ exposure (10 μM, 24 h) using dihydroethidium (DHE), a fluorescent probe sensitive to superoxide radicals. Scale bar=20 μm. (D) Spectrofluorometric detection and quantification of ethidium fluorescence intensity (arbitrary units) was expressed relative to untreated controls (CTL=1, white bar) (n=8). The data are presented as mean ±SEM obtained from at least three independent experiments performed in triplicates. Statistically significant differences are indicated by p** <0.01, p*** <0.001.