Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

A Series of Two-photon Absorption Pyridinium Sulfonate Inner Salts Targeting Endoplasmic Reticulum (ER), Inducing Cellular Stress and Mitochondria-Mediated Apoptosis in Cancer Cells

Sajid Hussain[a]‡, Wei Du[b]‡, Mingzhu Zhang[b], Bin Fang[b], Guocui Zhang[b], Su rina[a], Nan Keqian[a], Qiong Zhang[b], Xiaohe Tian*[a], Yan Chen*[a], YupengTian*[b]

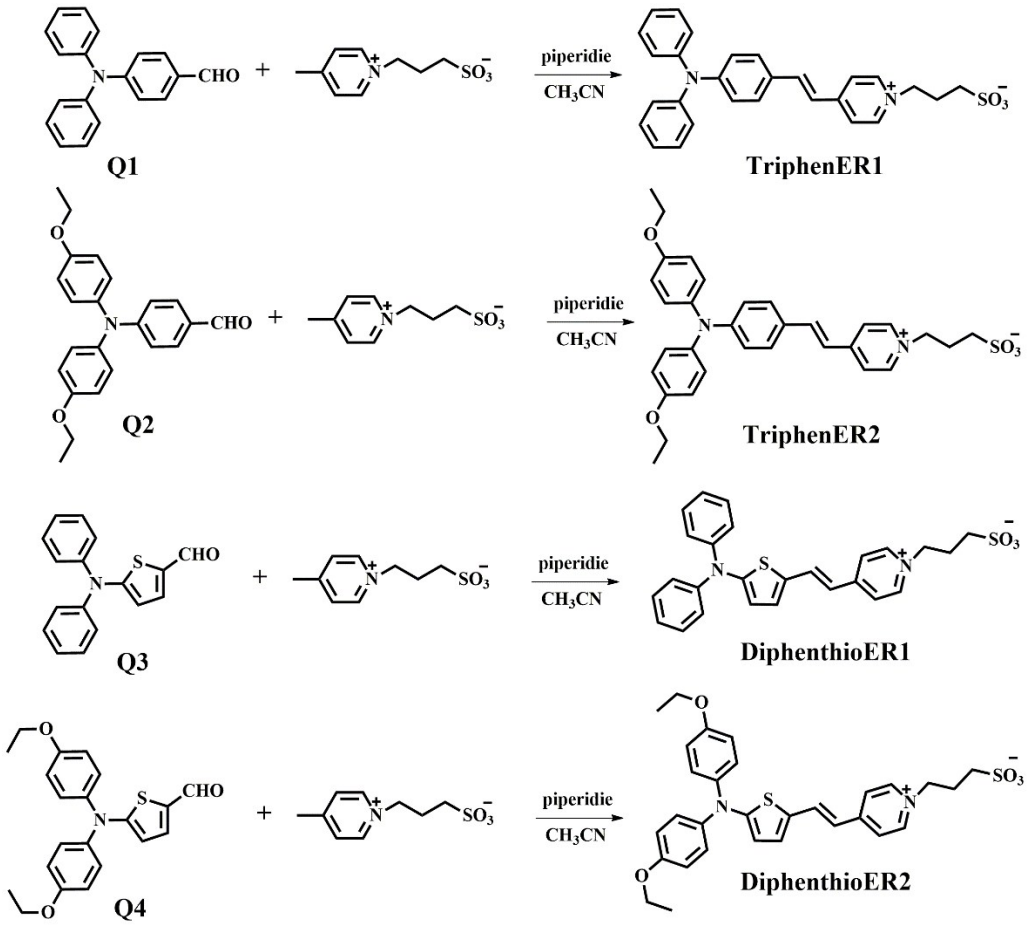
[a] School of Life Science, Anhui University, Hefei 230039, China

[b] Department of Chemistry, Key Laboratory of Functional Inorganic Material Chemistry of Anhui Province, Anhui University, Hefei 230039, P. R. China

* Corresponding author: xiaohe.t@ahu.edu.cn; chenyan@ahu.edu.cn; yptian@ahu.edu.cn

‡ These authors contributed equally to this work.

Supporting Information



Scheme S1. The synthesis routes of **TriphenER1-2** and **DiphenthioER1-2**.

Table S1. Crystal data collections and structure refinements of **DiphenthioER2**

Empirical formula	$C_{30}H_{32}N_2O_5S_2$
CCDC	1543839
Formula weight	564.70
Crystal system	Monoclinic
Space group	*C*2/*c*
A (Å)	46.394(10)
B (Å)	11.499(3)
C (Å)	11.4990(8)
β(°)	104.350(3)
Volume (Å^3)	5943(2)
Z	8
D_c(mg m^{-3})	1.302
μ (mm^{-1})	0.224
Reflns. Collected	14395
Reflns. Unique	4136
Parameters	363
Goodness-of-fit on F^2	1.050
R_1, wR_2 ([$I \geq 2\sigma(I)$])	$R_1 = 0.0616$, $wR_2 = 0.1283$
R_1, wR_2 (*all data*)	$R_1 = 0.1325$, $wR_2 = 0.1827$

Table S2. Selected bond lengths (Å) and angles (º) for**DiphenthioER2**

Bond	Å	Angle	º
S(2)- C(14)	1.738(5)	O(2)- S(1)- O(1)	114.0(3)
O(5)- C(26)	1.381(6)	C(14)- N(3)-C(15)	121.8(4)
C(16)- C(15)	1.369(7)	C(15)- N(3)-C(23)	118.6(4)

C(20)- C(19)	1.363(8)	C(2)- C(1)-S(1)	115.6(4)
C(26)- C(25)	1.375(8)	C(14)- N(3)- C(23)	119.2(4)
C(6)- C(5)	1.377(7)	C(12)- C(11)- S(2)	110.7(4)
C(3)- C(2)	1.507(8)	C(14)- S(2)- C(11)	91.0(3)

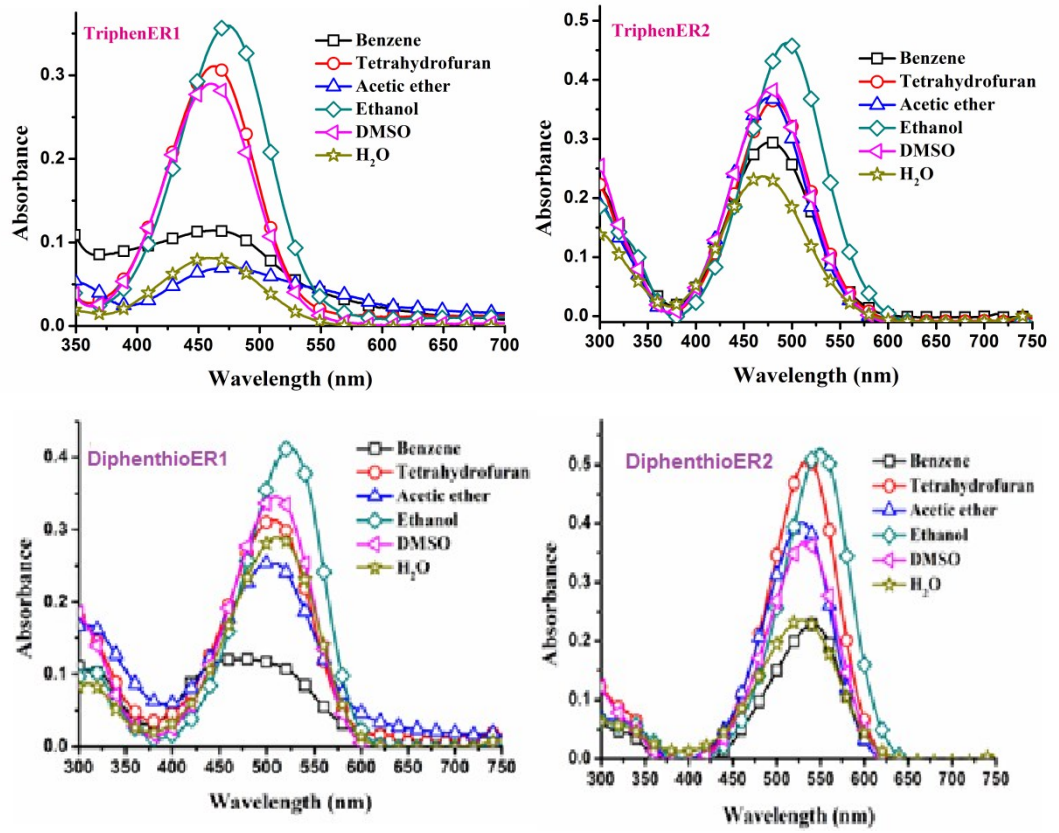
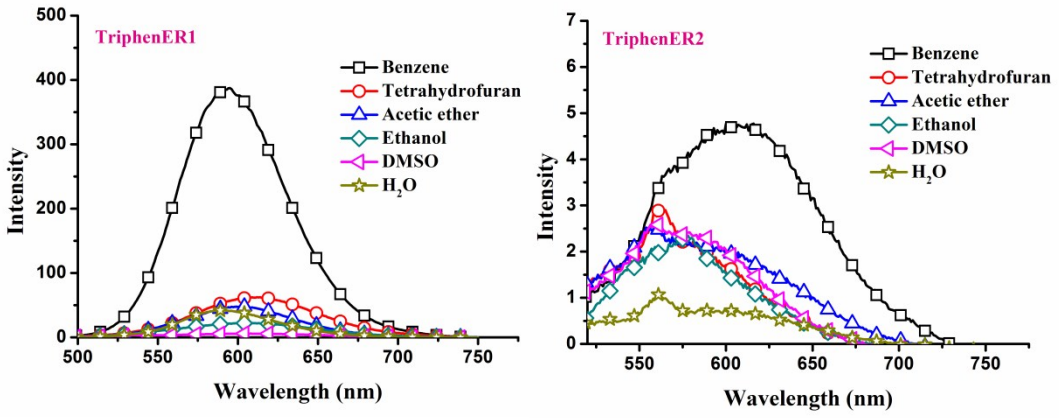


Figure S1. UV-vis absorption spectra of **TriphenER1-2** and **DiphenthioER1-2** in different solvents (c=10 μM).



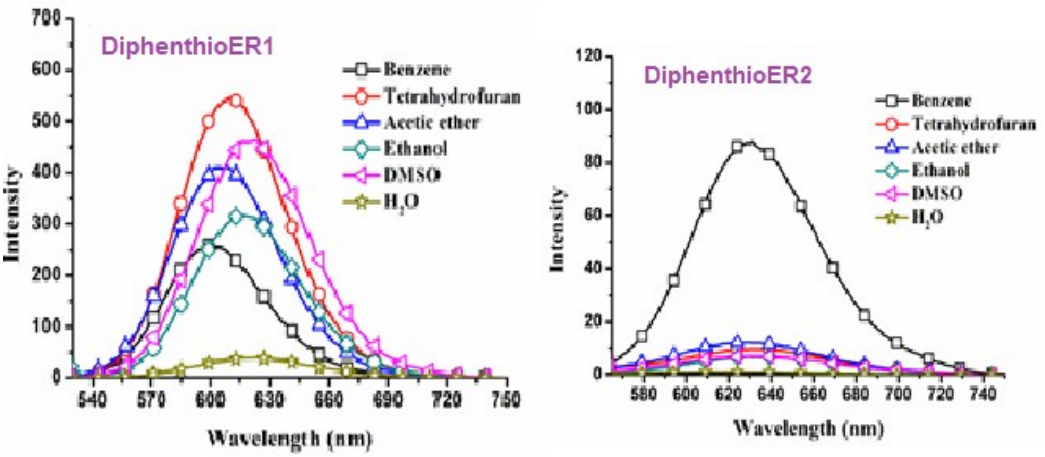


Figure S2. One-photon fluorescence spectra of **TriphenER1-2** and **DiphenthioER1-2** in different solvents (c=10 μM).

Table S3. Optical physics data of **TriphenER1-2** and **DiphenthioER1-2**

compound	Solvents	λ^{abs}_{max} (nm)[a]	ε^{b}_{max}	λ^{SPEF}_{max} (nm)[c]	Δν (nm)[e]
TriphenER1	Benzene	467	1.1	590	123
	Tetrhydrofuran	463	3.1	599	136
	Acetic ether	472	0.69	595	123
	Ethanol	473	3.6	603	130
	DMSO	460	2.9	612	152
	H_2O	459	0.84	606	147
TriphenER2	Benzene	475	2.94	595	120
	Tetrhydrofuran	479	3.61	613	134
	Acetic ether	478	3.74	603	125
	Ethanol	492	4.64	606	114
	DMSO	478	3.84	607	129
	H_2O	471	2.37	589	118
DiphenthioER1	Benzene	486	1.23	615	129
	Tetrhydrofuran	506	3.12	562	56
	Acetic ether	504	2.56	588	84

	Ethanol	514	4.14	578	64
	DMSO	509	3.42	574	65
	H_2O	513	2.89	599	86
Diphenthio ER2	Benzene	541	2.23	631	90
	Tetrhydrofuran	533	5.07	634	101
	Acetic ether	519	4.02	625	106
	Ethanol	549	5.30	628	79
	DMSO	532	3.70	633	101
	H_2O	526	2.37	619	93

[a]Peak position of the longest absorption band. [b] Molar absorbance in 10^4 mol^{-1} L cm^{-1}. [c]Peak position of one-photon fluorescence spectra, excited at the absorption maximum.[d]Fluorescence lifetime(ns). [e] Stokes' shift in nm. [f] Quantum yields determined by using fluorescein as standard

Table S4. Excitation energies, corresponding wavelengths, oscillator strengths and major contribution for **TriphenER1-2** and **DiphenthioER1-2**. (H: HOMO, L: LUMO)

Compound	ΔE(eV)[a]	λ (nm)[b]	λ (nm)[c]	Oscillator strengths	Nature of the transition
TriphenER1	2.7584	449	455	0.0059	120(H-4)→125(L+1)
TriphenER2	2.6698	481	474	0.0003	156(H)→159(L+2)
DiphenthioER1	2.3900	519	520	1.2443	125(H)→126(L)
DiphenthioER2	2.3019	539	532	1.2411	149(H)→150(L)

[a] The energy gap of the single-photon absorption band. [b]Theoreticalpeak position of the linear absorption band. [c]Experimental peak position of the linear absorption band.

Table S5. The optimum wavelength of pulse laser and two-photon absorption cross sections (σ) of **TriphenER1-2** and **DiphenthioER1-2** in PBS buffer (c= 0.1 mM).

Compound	λ (nm)[a]	σ (GM)[b]
TriphenER1	820	163
TriphenER2	820	568
DiphenthioER1	840	1779
DiphenthioER2	830	2023

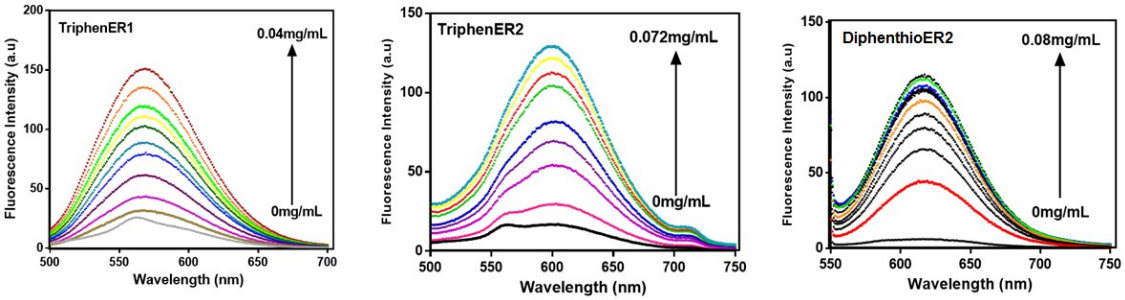


Figure S3. Changes of fluorescence spectra of **TriphenER1**, **TriphenER2** and **DiphenthioER2** with varying concentration of liposome

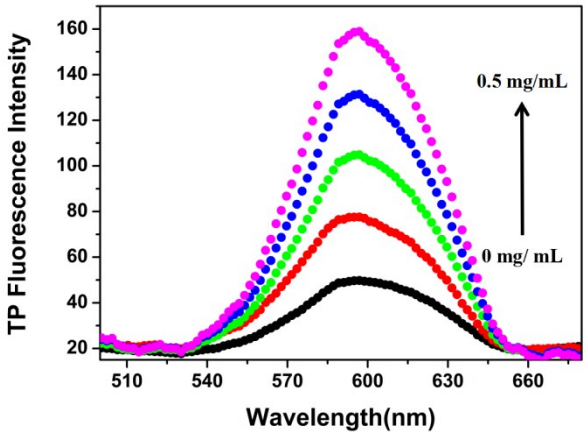


Figure S4. Changes of two-photon fluorescence spectra of **DiphenthioER1** with varying concentration of liposome. (λ_{ex} = 900 nm, λ_{em} = 610-640 nm).

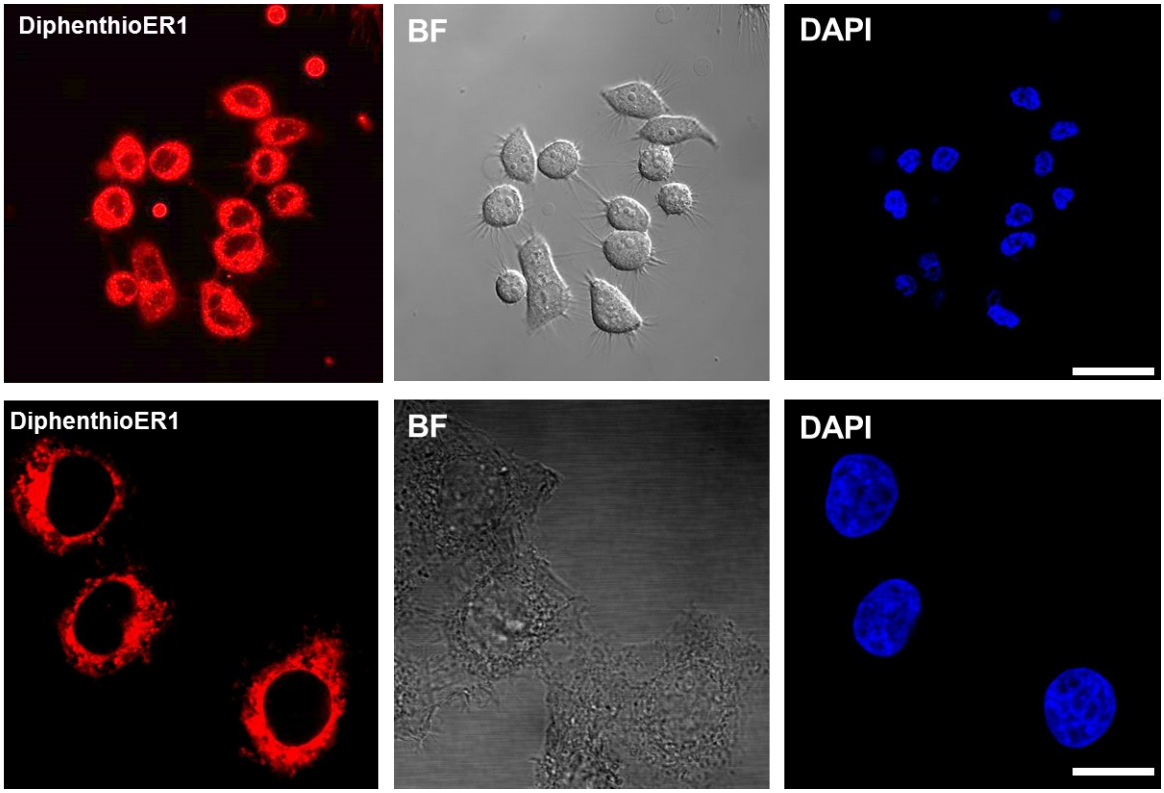


Figure S5. Confocal microscopy images of **DiphenthioER1** treated fixed cells (top) and live cells (bottom) after DAPI staining

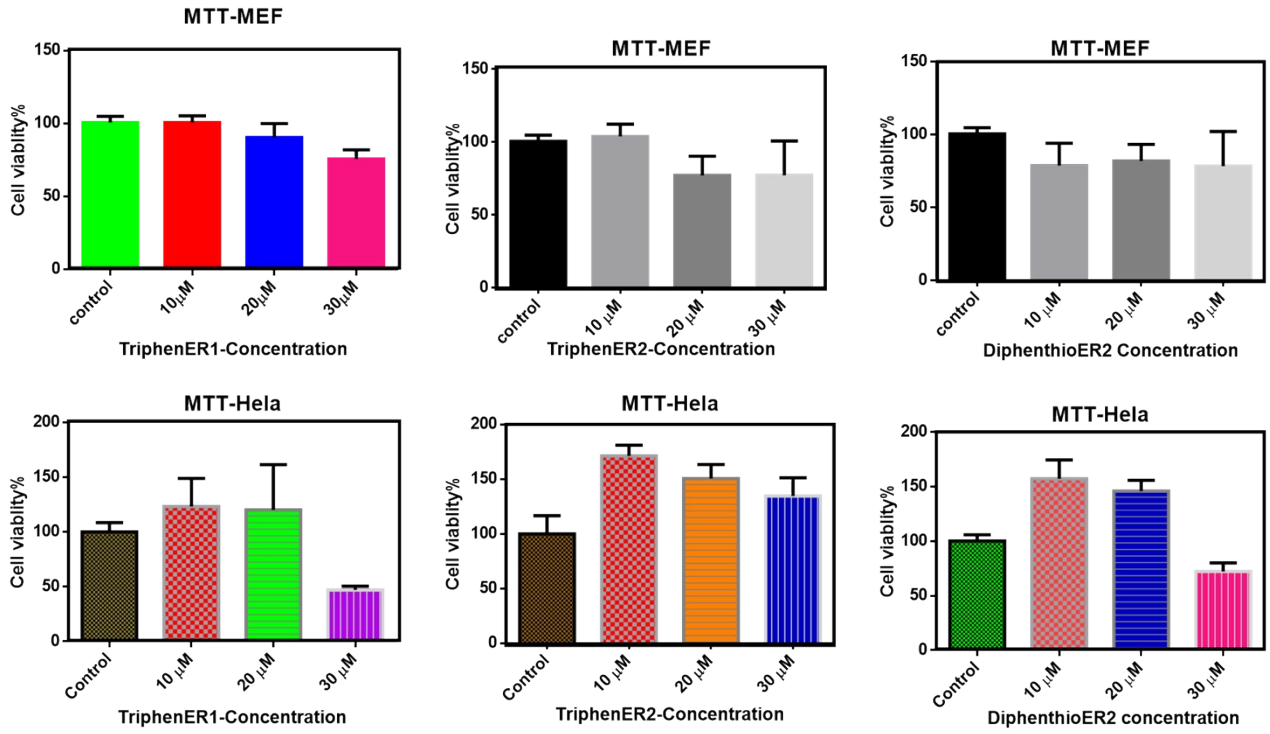


Figure S6. Effect of **TriphenER1**, **TriphenER2** and **DiphenthioER2** on percentage cell viability of normal cells (MEF) and cancer cell lines (HeLa)

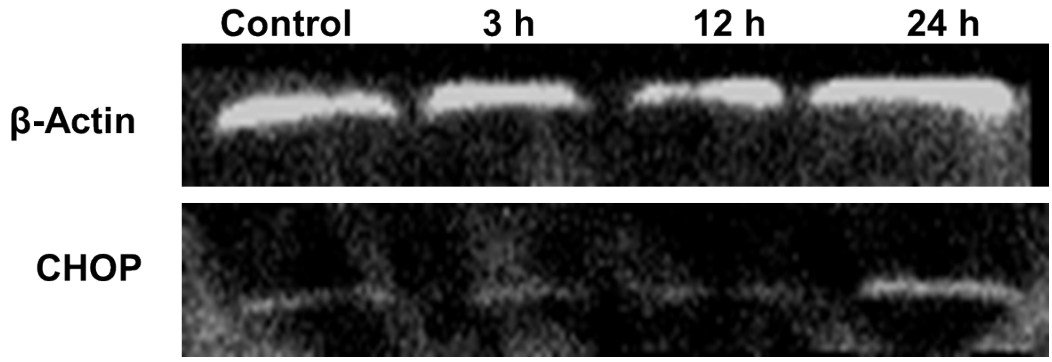


Figure S7. Western blot analysis for CHOP after treatment with **DiphenthioER1**

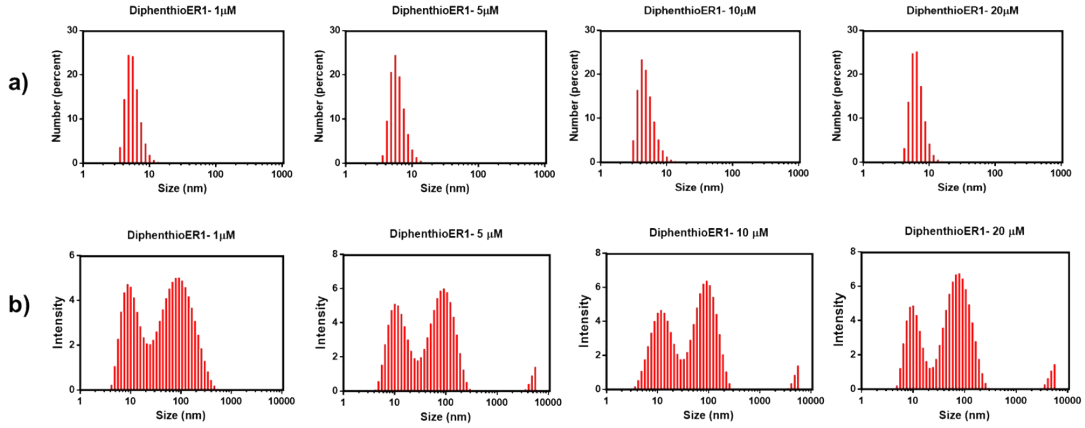


Figure S8. Particle size distribution of **DiphenthioER1** with varying concentrations (1-20 μM) in cell culture medium using Dynamic Light Scattering (DLS); a) Distribution by number; b) Distribution by intensity

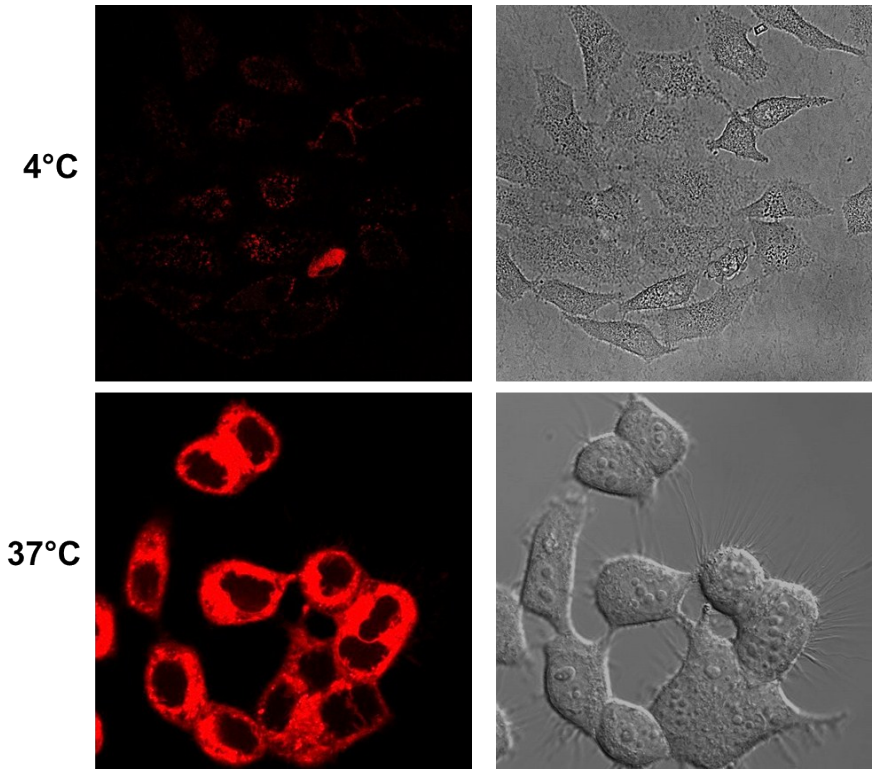


Figure S9. Temperature-dependent uptake of **DiphenthioER1**. HepG2 cells incubated with **DiphenthioER1** (10 μM for 30 min) at either 4 °C (top) or 37 °C (bottom). Same microscopy settings used for each incubation condition.