Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Supramolecular self-assembly of triazine-based small molecule: targeting endoplasmic reticulum in cancer cells

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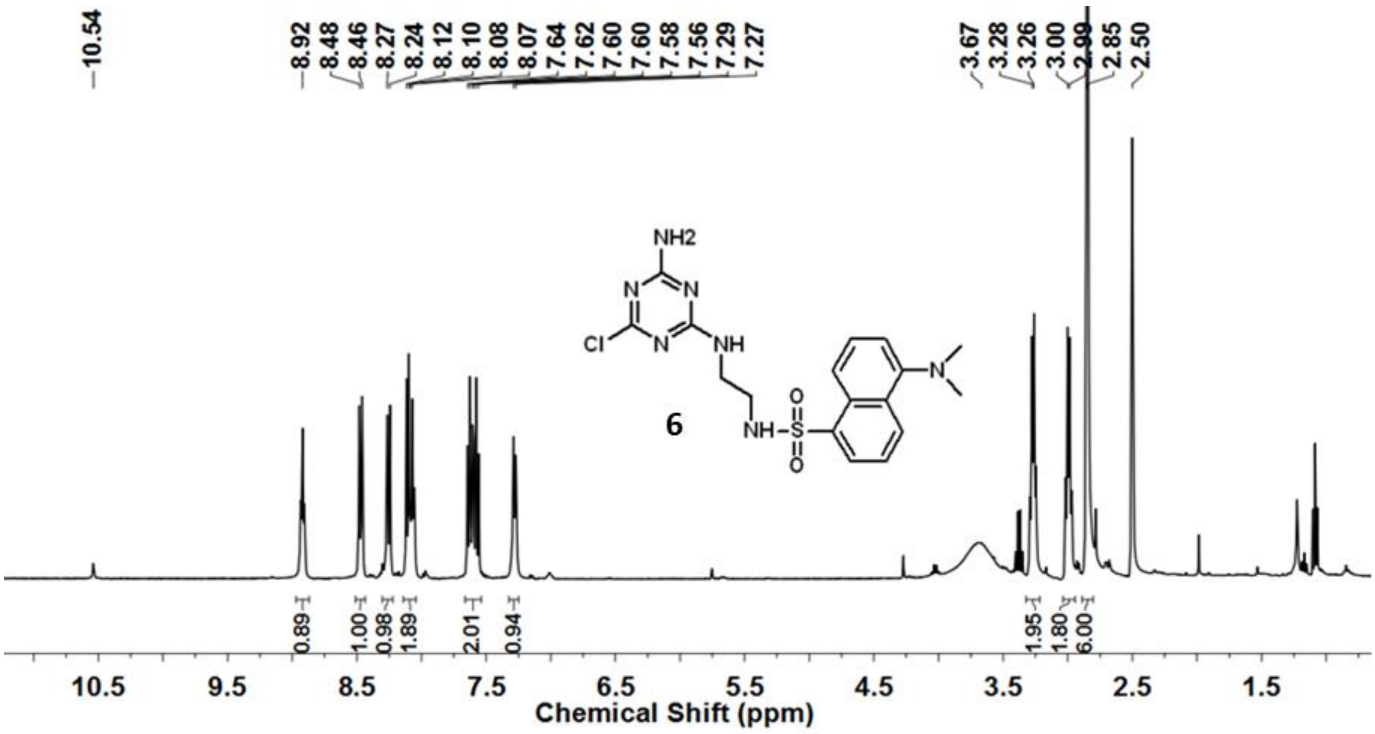

Fig. S1: ^{1}H NMR spectra of compound **6**.

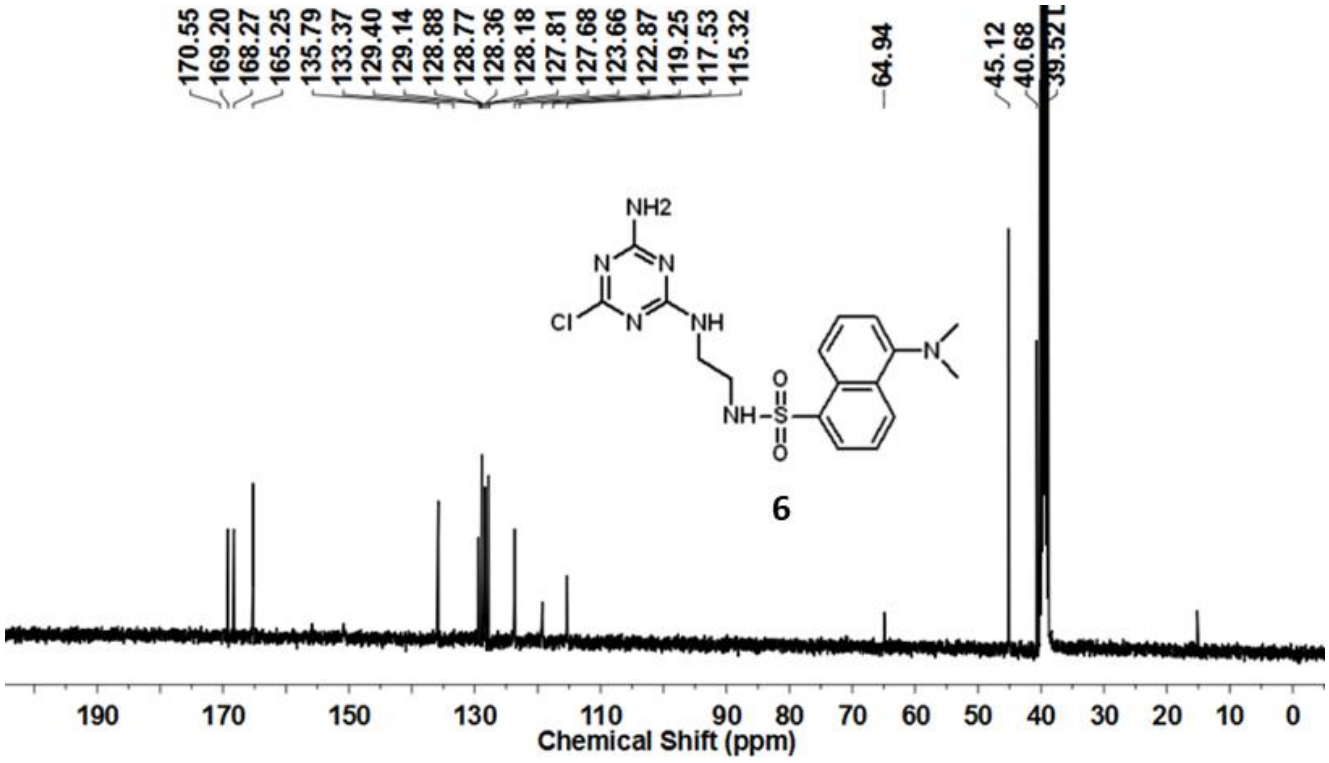

Fig. S2: ^{13}C NMR spectra of compound **6**.

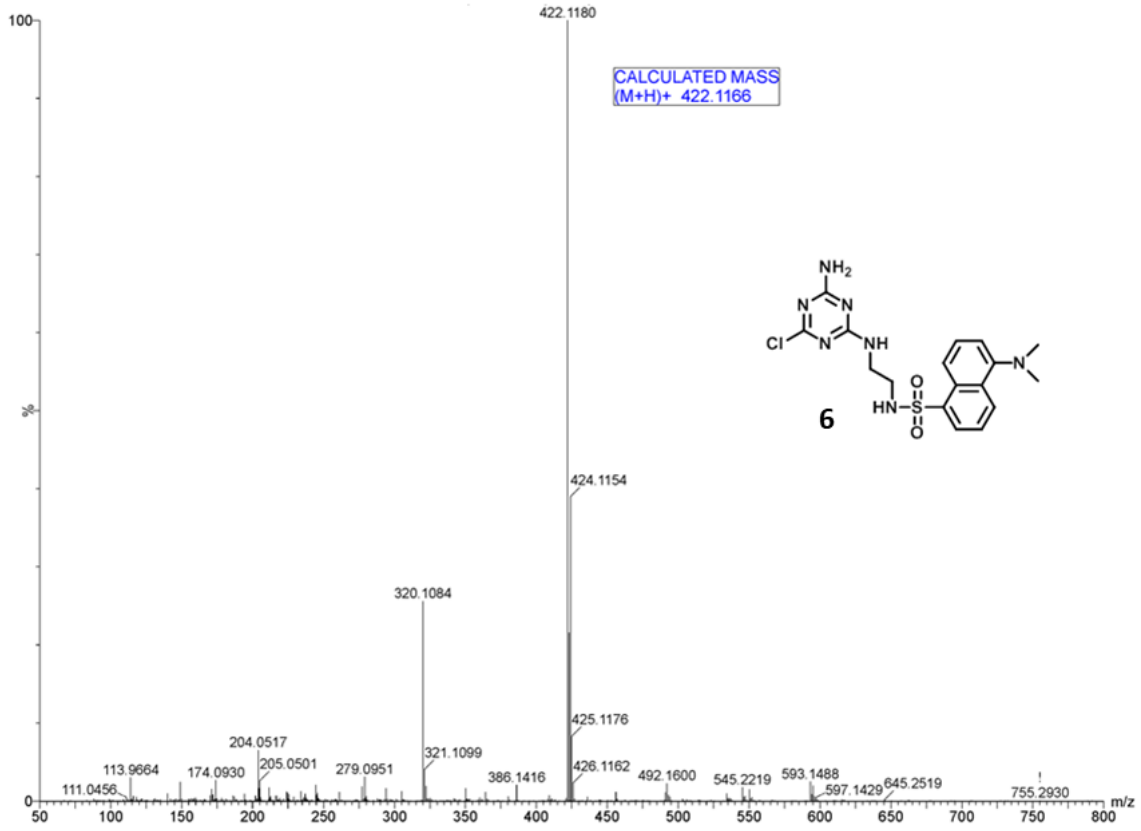

Fig. S3: HR-MS spectra of compound **6**.

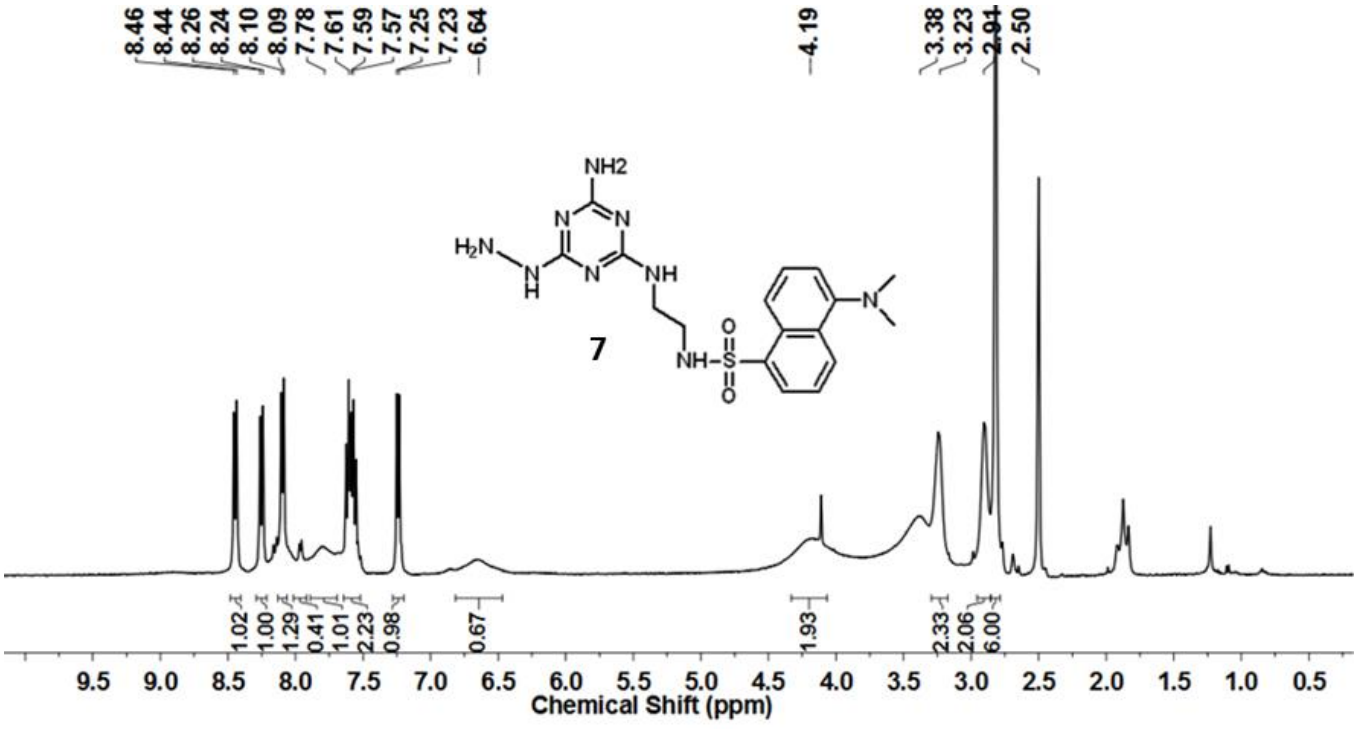

Fig. S4: ^{1}H NMR spectra of compound **7**.

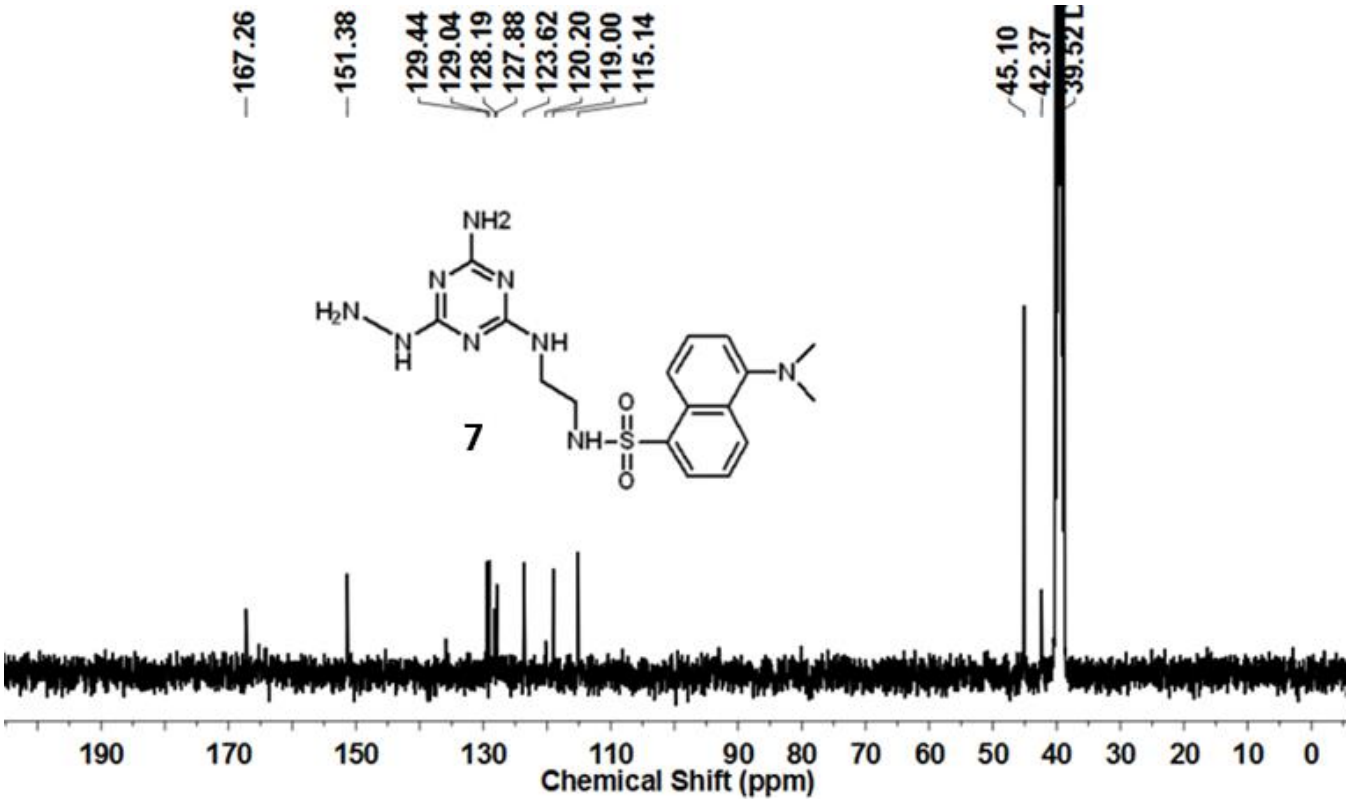

Fig. S5: ^{13}C NMR spectra of compound **7**.

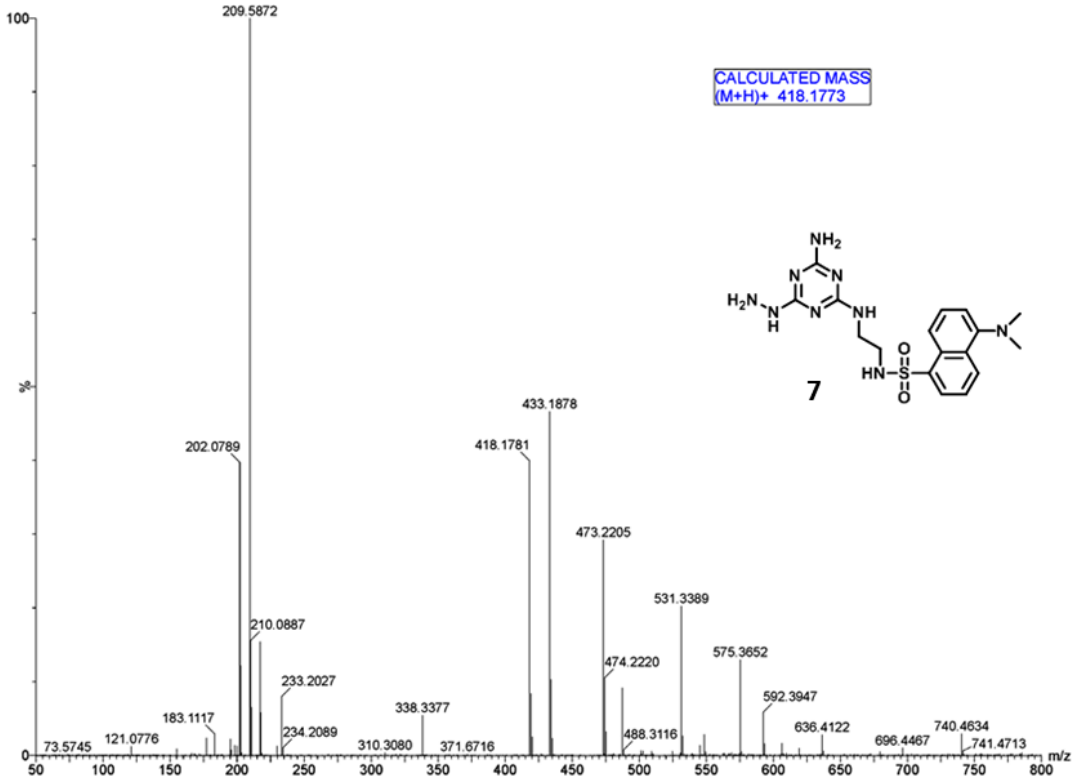

Fig. S6: HR-MS spectra of compound **7**.

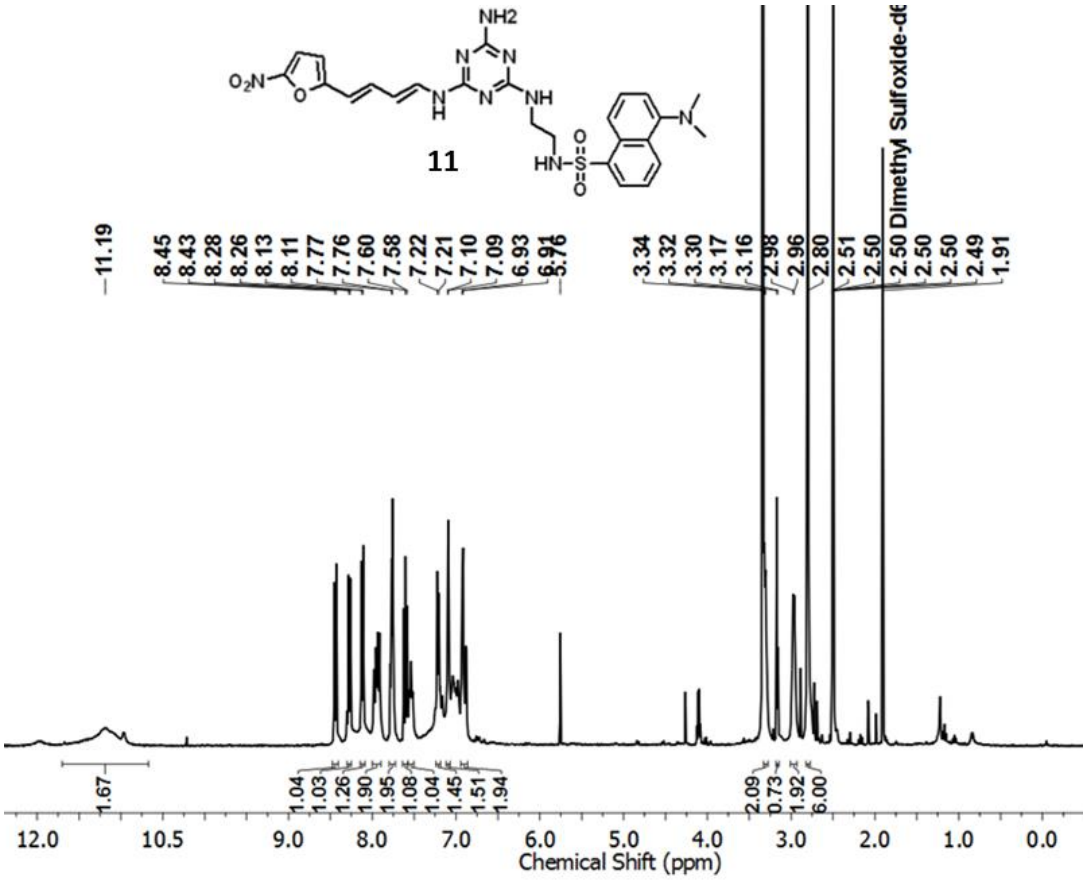

Fig. S7: ^{1}H NMR spectra of compound **11**.

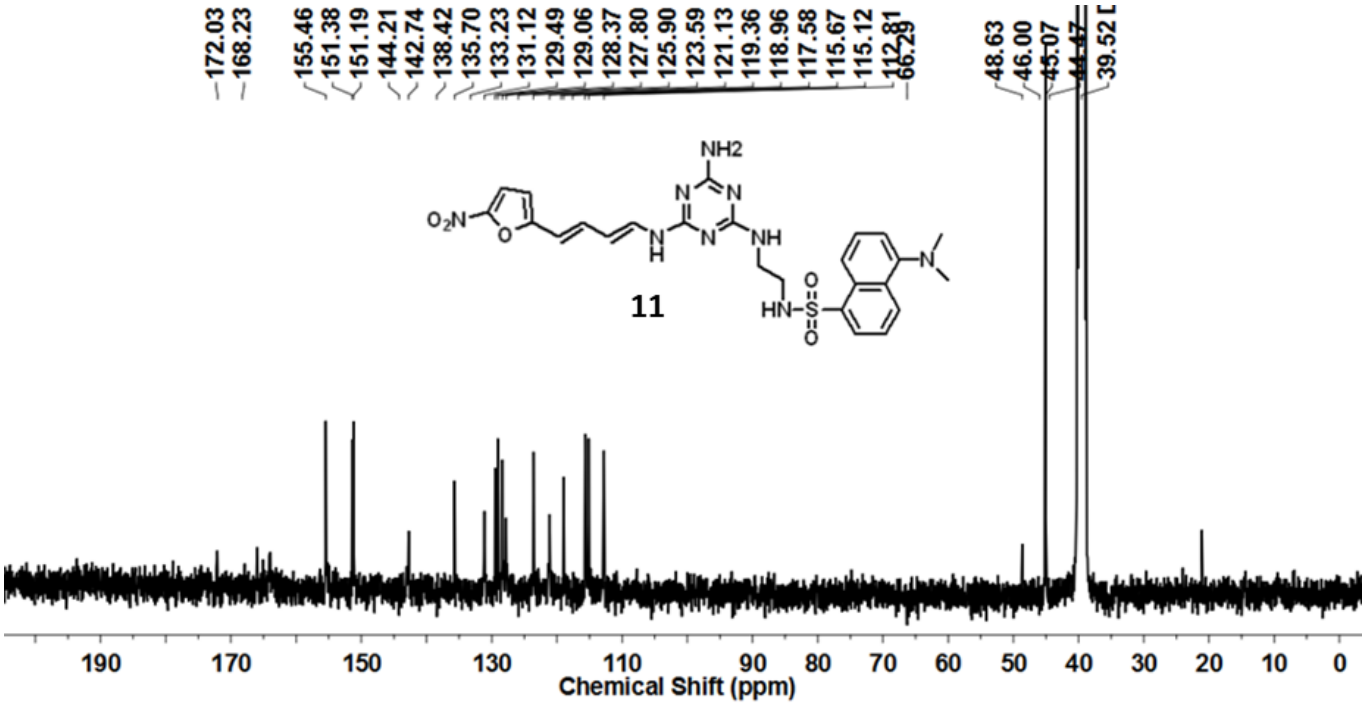

Fig. S8: ^{13}C NMR spectra of compound **11**.

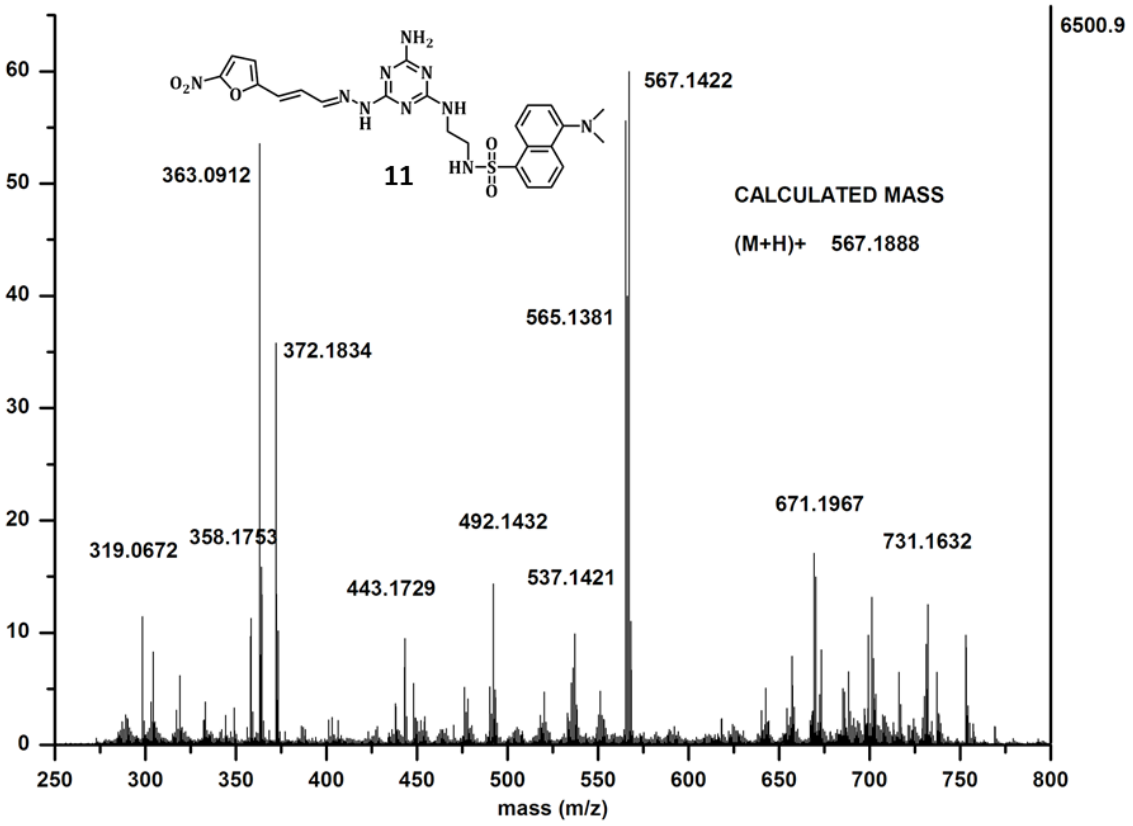

Fig. S9: HR-MS spectra of compound **11**.

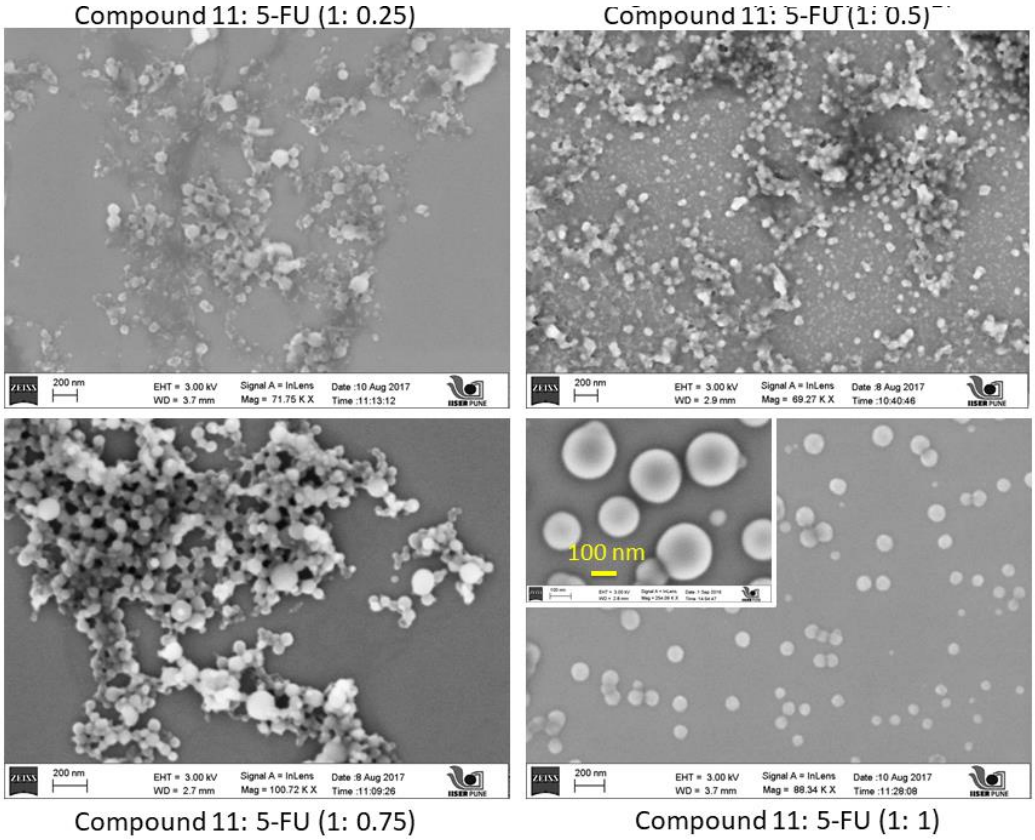

Fig. S10: FESEM spectra of mixture of compound **11** and 5FU in different ratios to visualize the self-assembly.

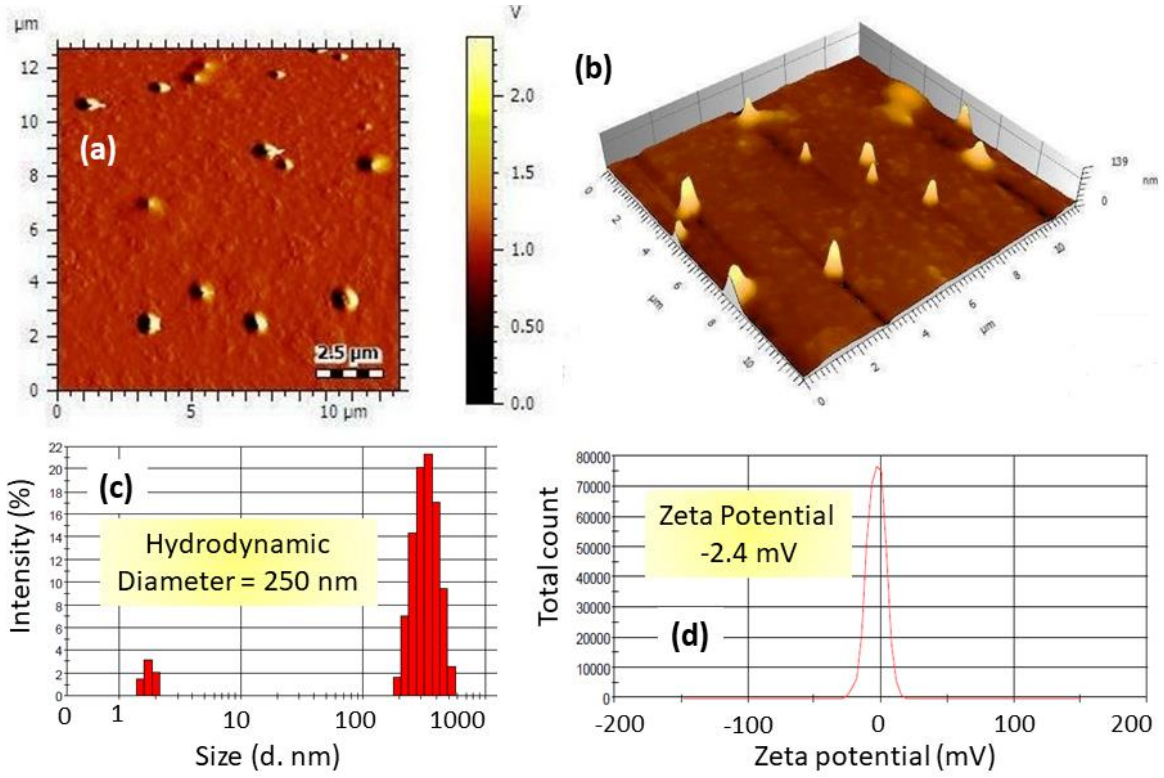

Fig. S11: (a,b) 2D and 3D AFM images of ER-NPs respectively. (c, d) hydrodynamic diameter and zeta potential of ER-NPs determined by DLS.

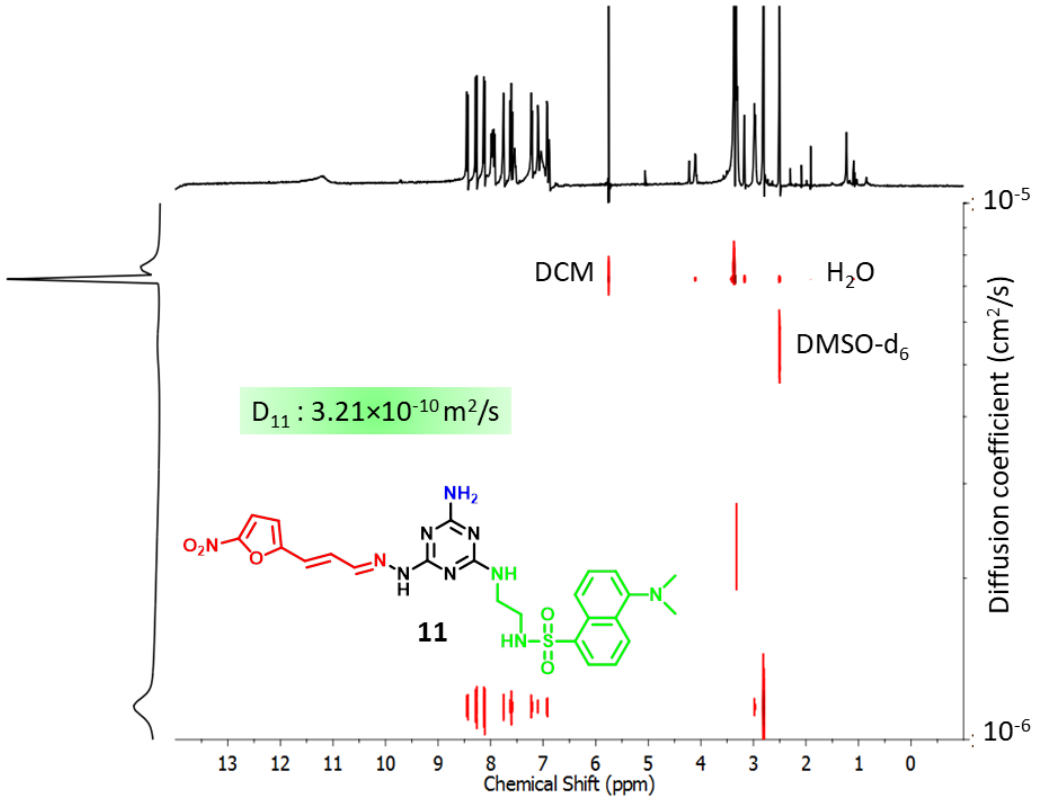

Fig. S12: DOSY spectra of compound **11**.

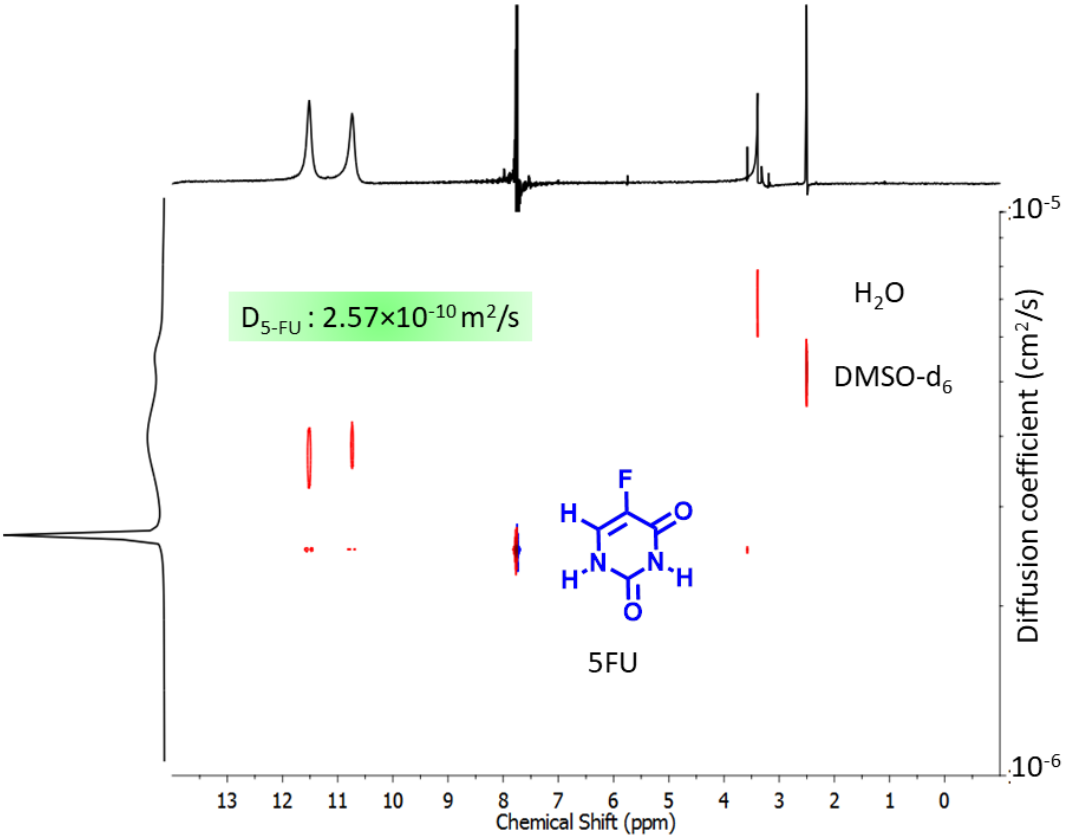

Fig. S13: DOSY spectra of 5-FU.

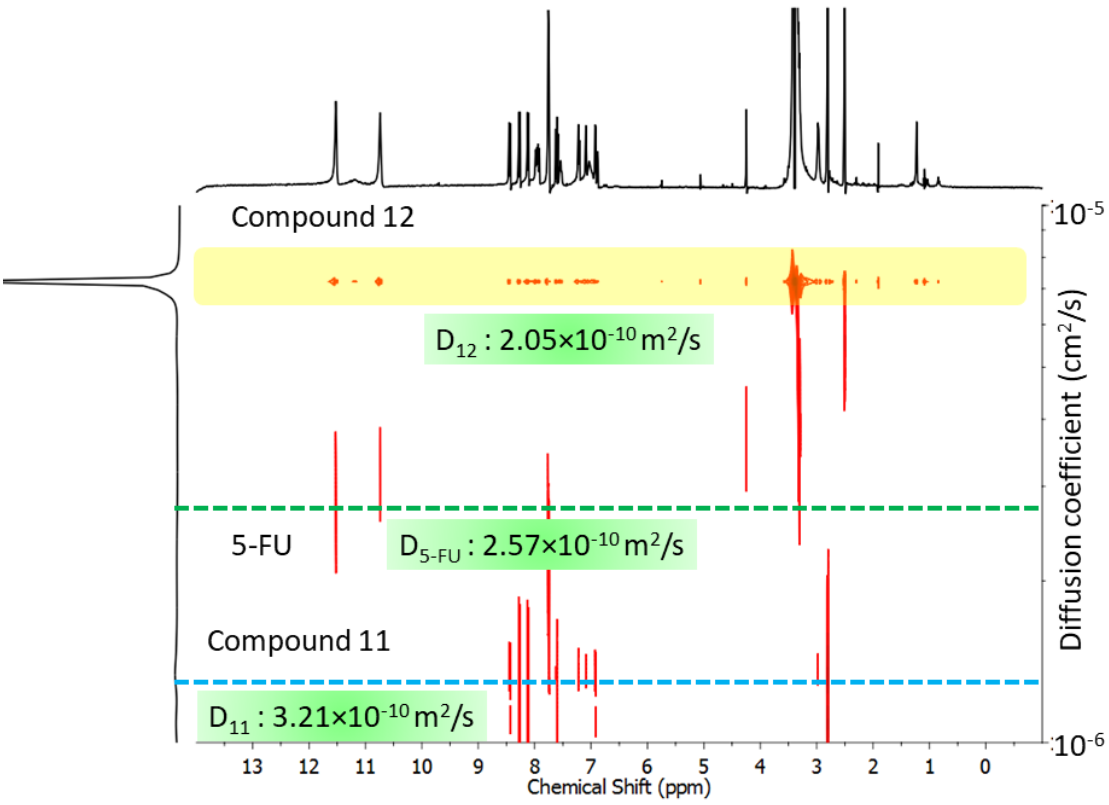

Fig. S14: Comparison of diffusion coefficients of compound **11**, compound **12** and 5-FU by DOSY spectra.

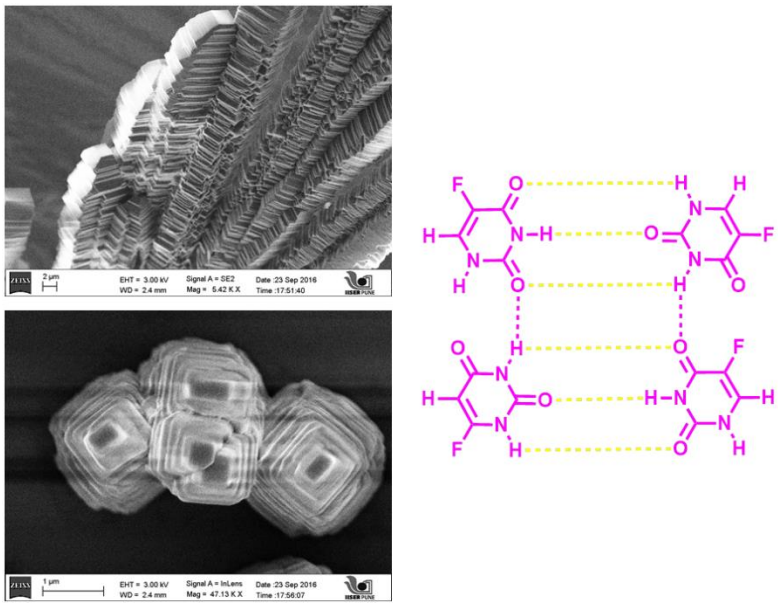

Fig. S15: FESEM images and proposed H-bonding in 5-FU.

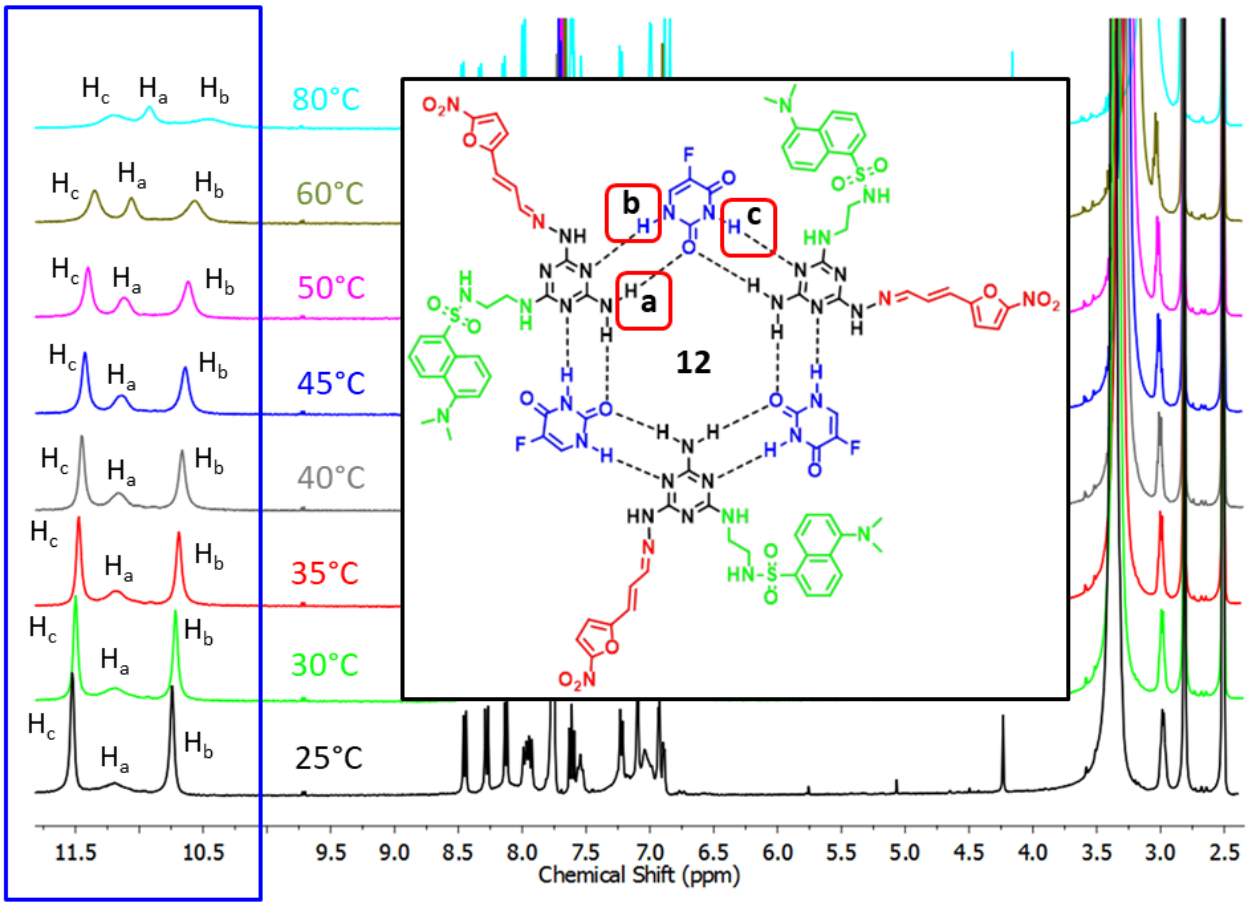

Fig. S16: Temperature dependent ^{1}H NMR spectra of self-assembled hexameric rosette structure **12**.

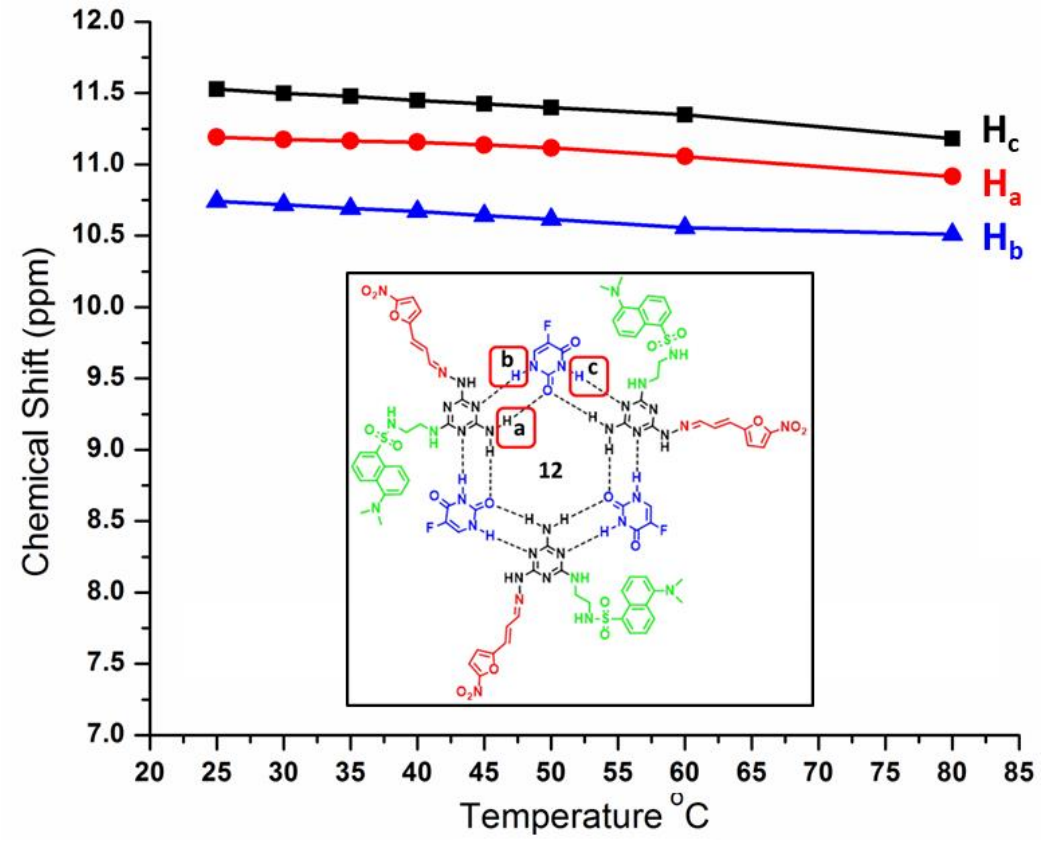

Fig. S17: Plot of temperature dependent ^{1}H NMR chemical shift of H_a, H_b and H_c in self-assembled hexameric rosette structure **12**.

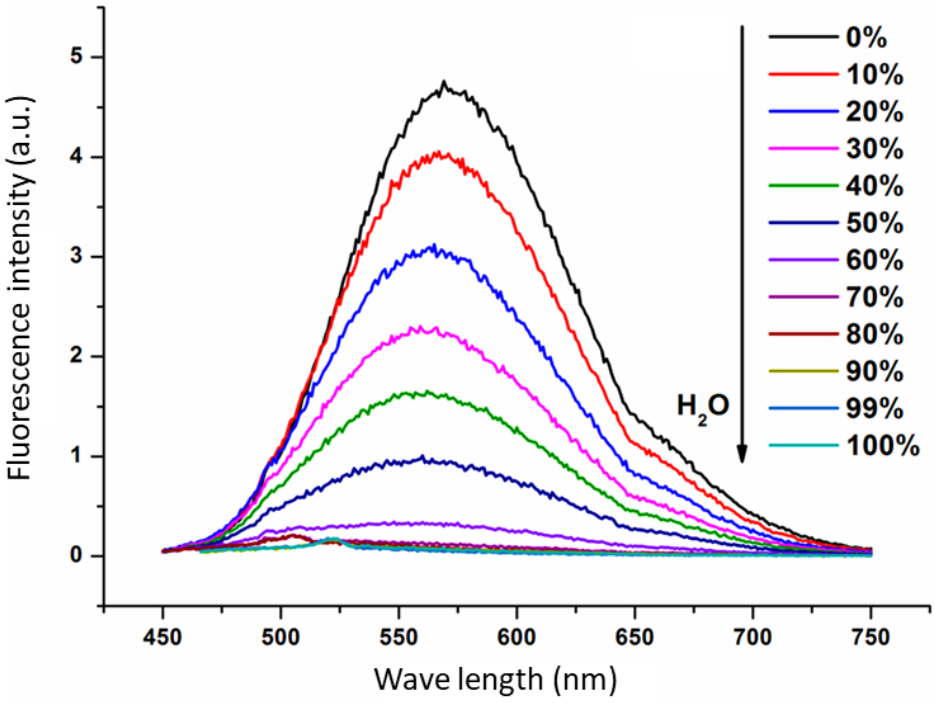

Fig. S18: Quenching of fluorescence emission intensity of dansyl group at λ_{max} = 575 nm in ER-NP upon self-assembly in water.

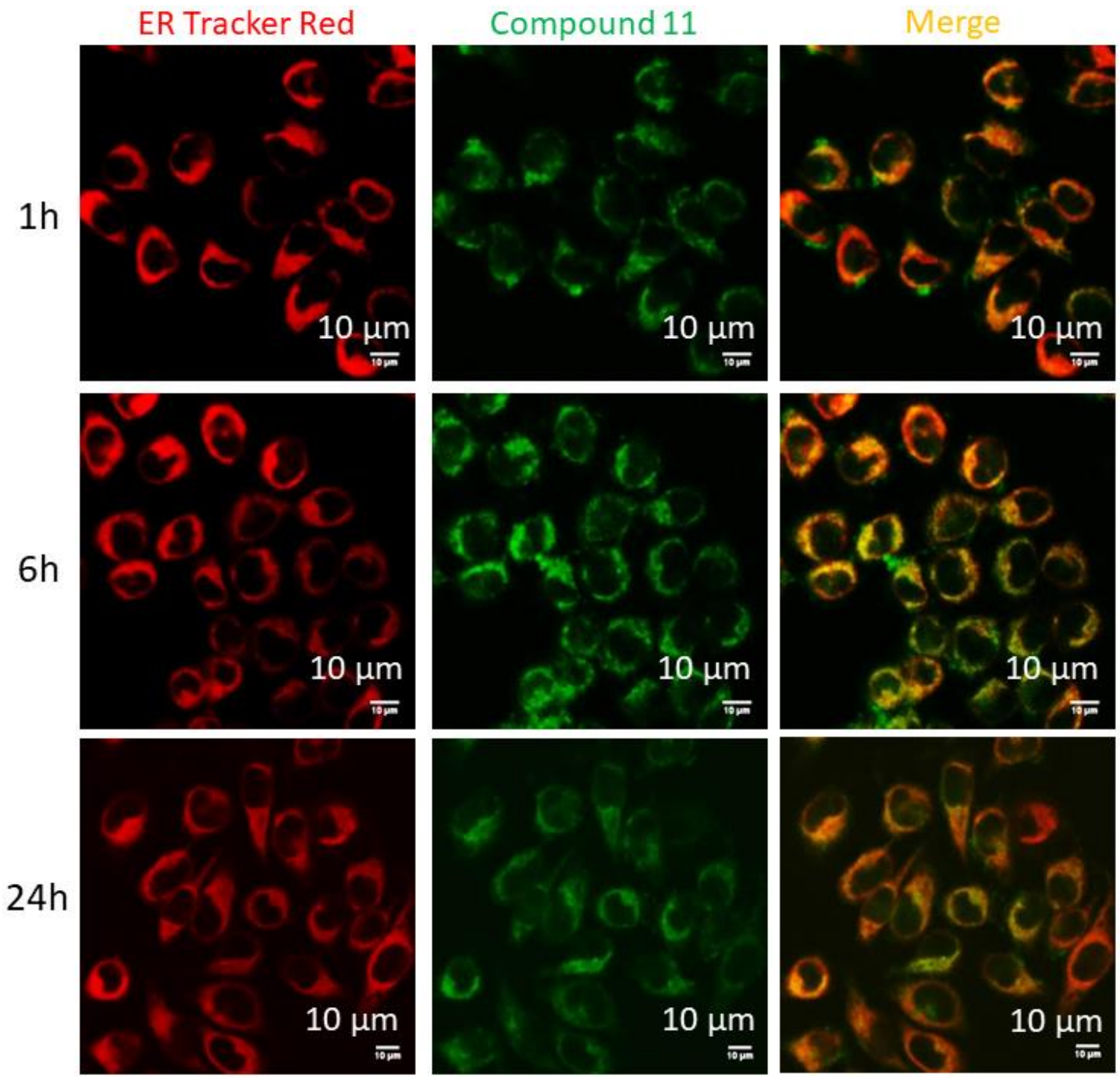

Fig. S19: Confocal laser scanning microscopy (CLSM) images of compound **11** in HeLa cells in different time points (1 h, 6 h and 24 h). ER is stained by ER-Tracker Red. Scale bar = 10 µm.

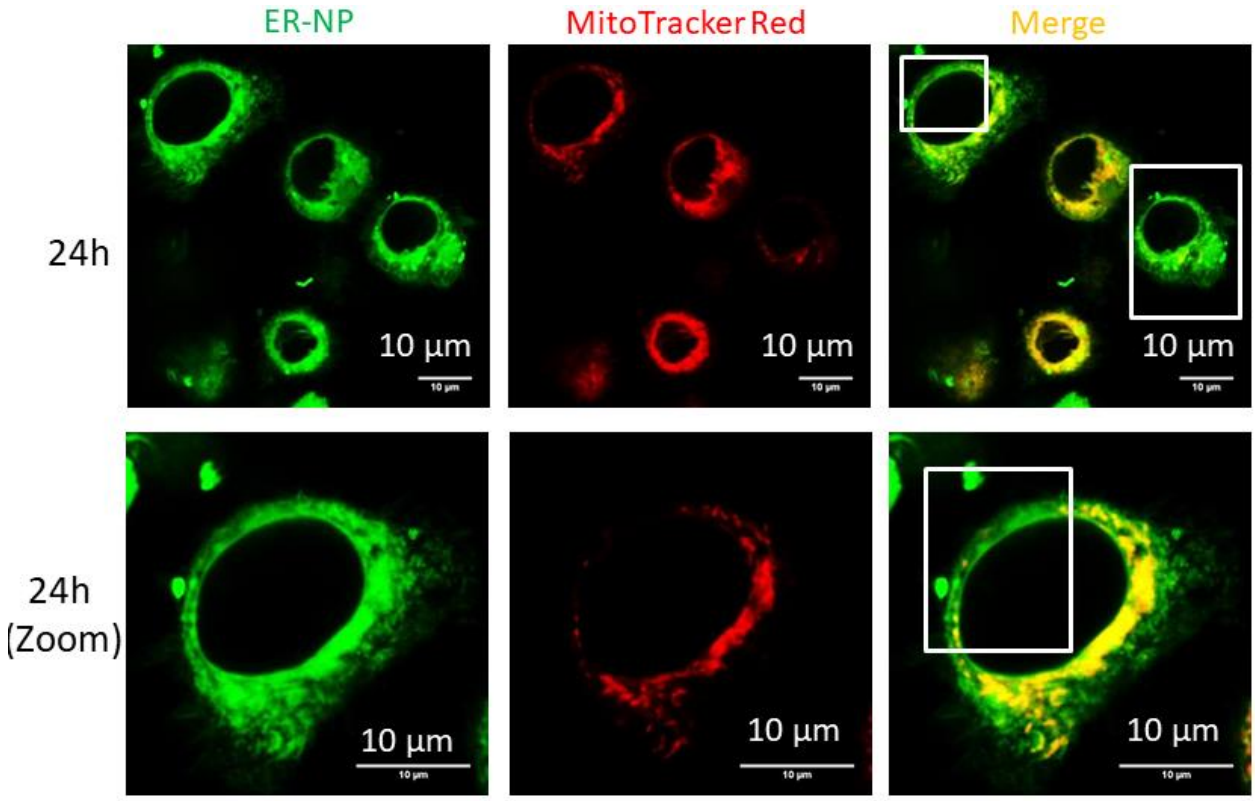

Fig. S20: CLSM images of ER-NPs in HeLa cells at 24 h. Mitochondria were stained with MitoTracker Deep Red dye. Scale bar = 10 µm.

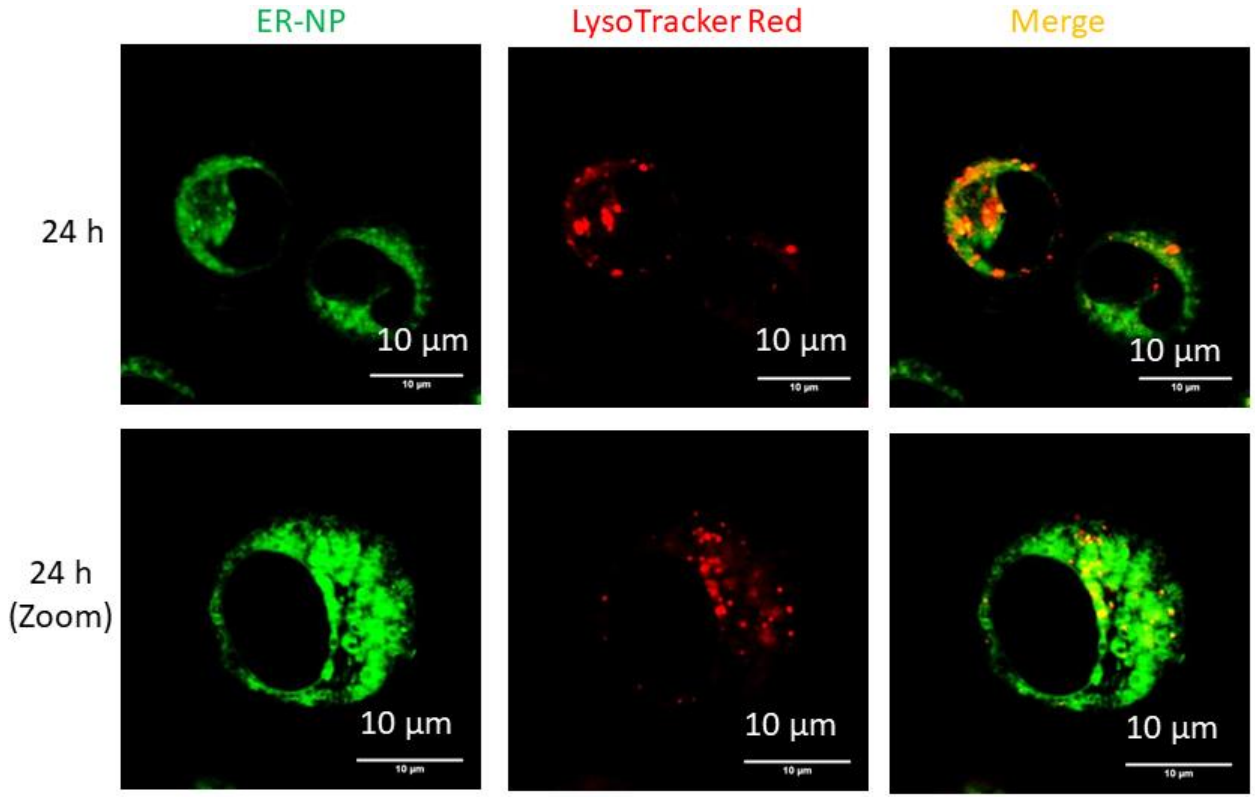

Fig. S21: CLSM images of ER-NPs in HeLa cells at 24 h. Lysosomal compartments were stained with LysoTracker Red dye. Scale bar = 10 µm.

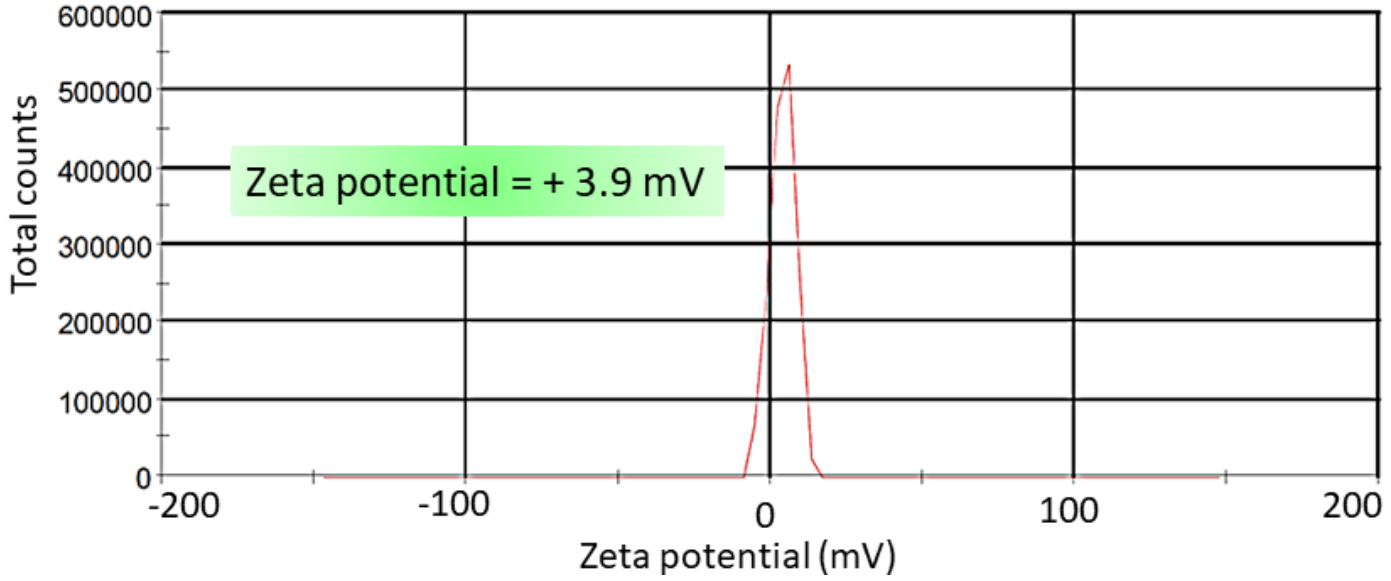

Fig. S22: Zeta potential of ER-NPs in pH = 5.5 solution determined by DLS.

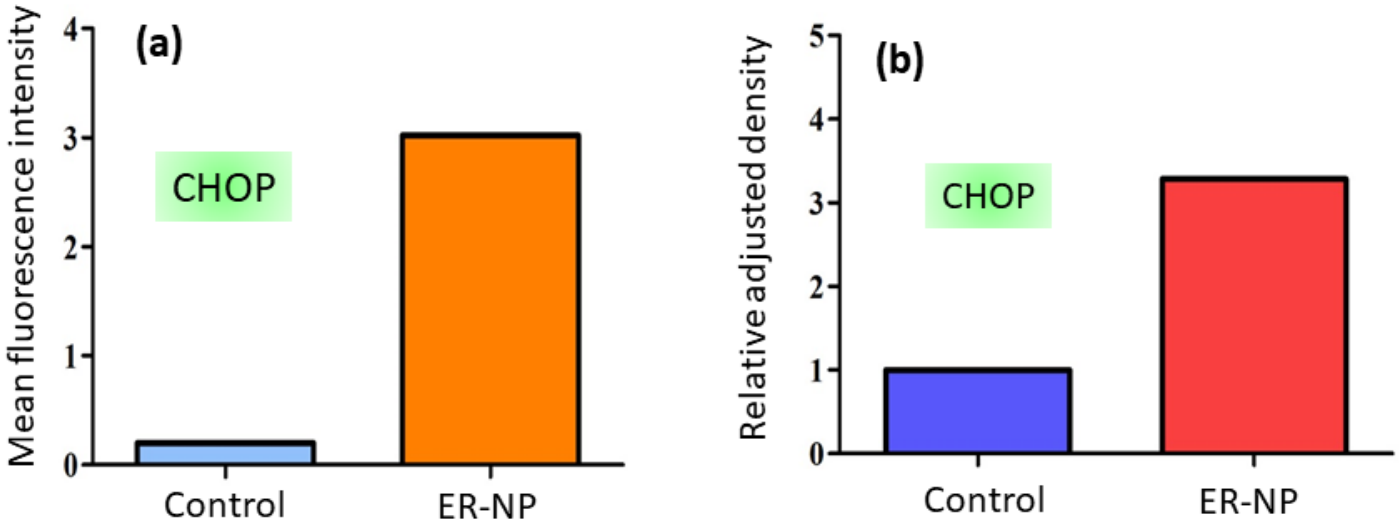

Fig. S23: (a, b) Quantification of expression of CHOP in HeLa cells after treatment with ER-NPs by CLSM images and Western blot analysis respectively.

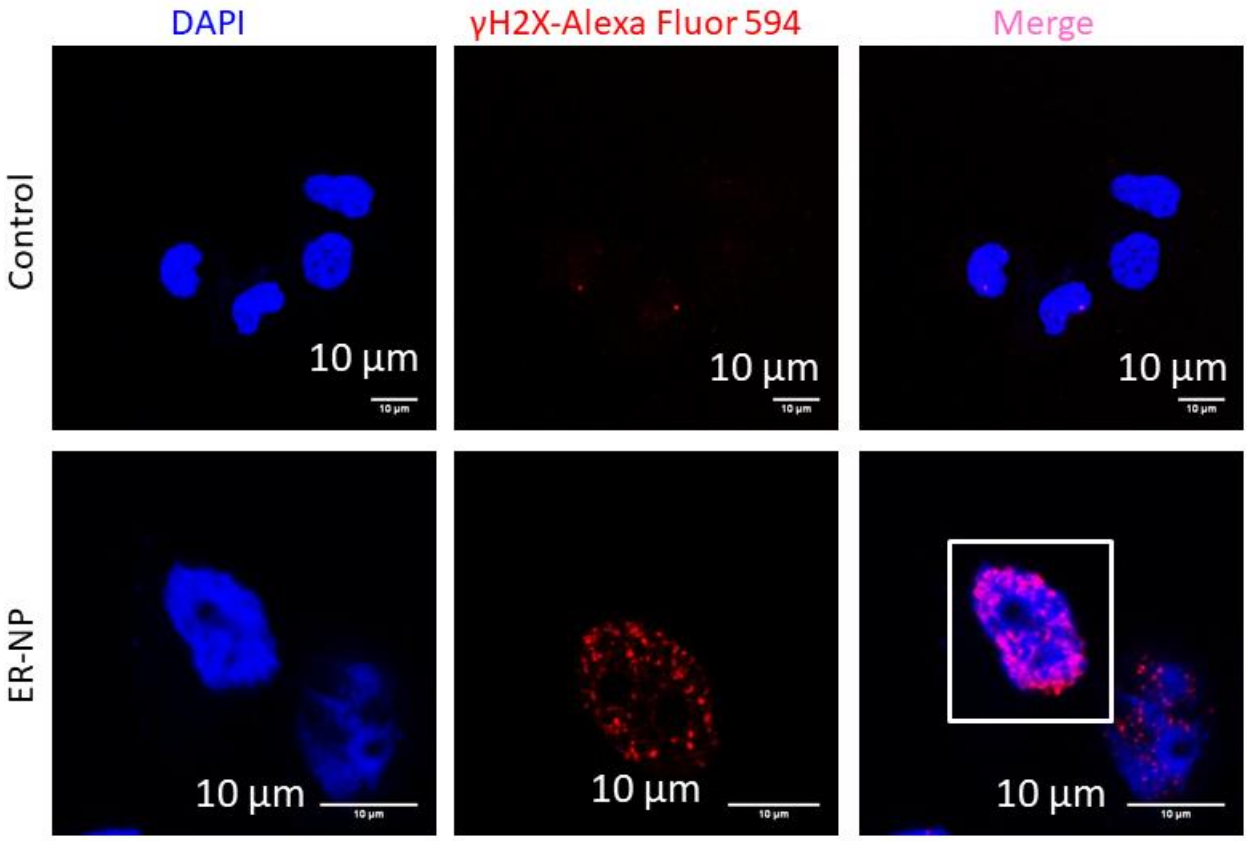

Fig. S24: CLSM images of HeLa cells after treatment with ER-NPs for 24 h. Nuclei were stained with blue fluorescent DAPI and γH2AX was stained with red fluorescently labeled Alexa Fluor 594 antibody to show DNA damage. Scale bar = 10 µm.

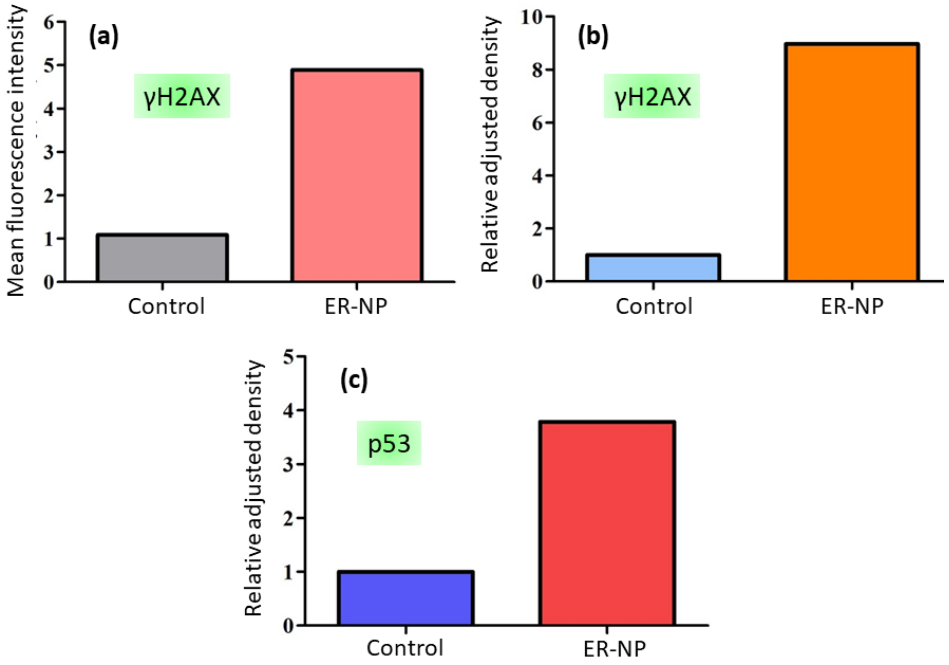

Fig. S25: (a,b) Quantification of γH2AX expression in HeLa cells after treatment with ER-NPs for 24 h determined from CLSM images and Western blot analysis respectively. (c) Quantification of p53 expression in HeLa cells after treatment with ER-NPs for 24 h, determined by Western blot analysis.

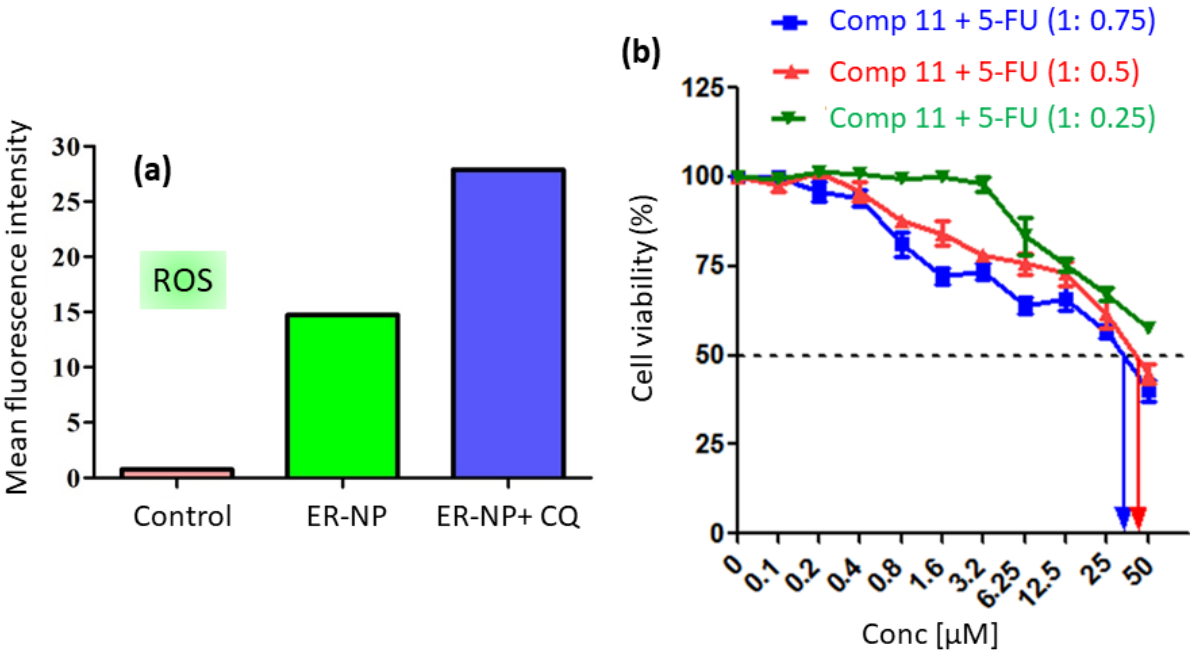

Fig. S26: (a) Quantification of ROS generated from CLSM images in HeLa cells after treatment with ER-NPs and ER-NP-CQ combination for 24 h. (b) Cell viability of HeLa cells after treatment with self-assembled materials obtained from compound 11 and 5-FU in different ratios (1: 0.75, 1: 0.5 and 1: 0.25) at 48 h post-incubation.

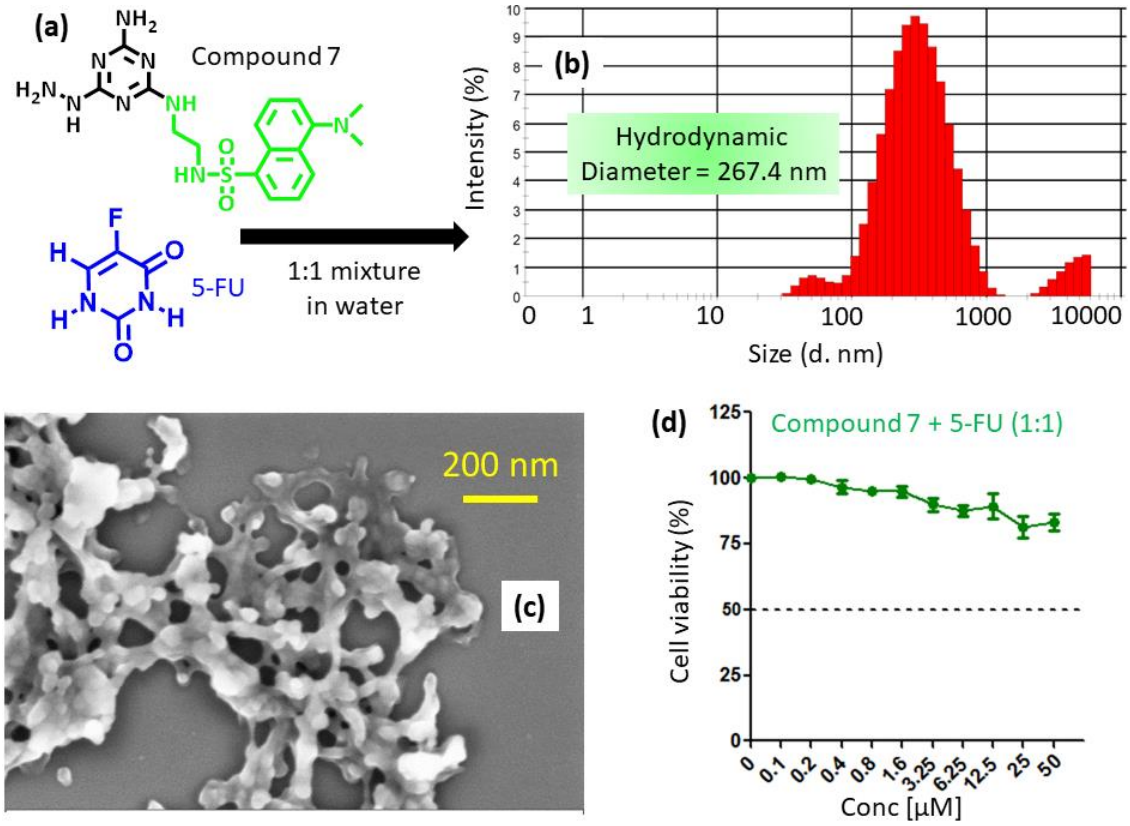

Fig. S27: (a) Scheme of mixing compound 7 and 5-FU in 1:1 ratio in water. (b,c) DLS and FESEM images of self-assembled material obtained by mixing compound 7 and 5-FU in 1:1 ratio in water. (d) Cell viability of HeLa cells after treatment with the self-assembled material obtained by mixing compound 7 and 5-FU in 1:1 ratio for 48 h.

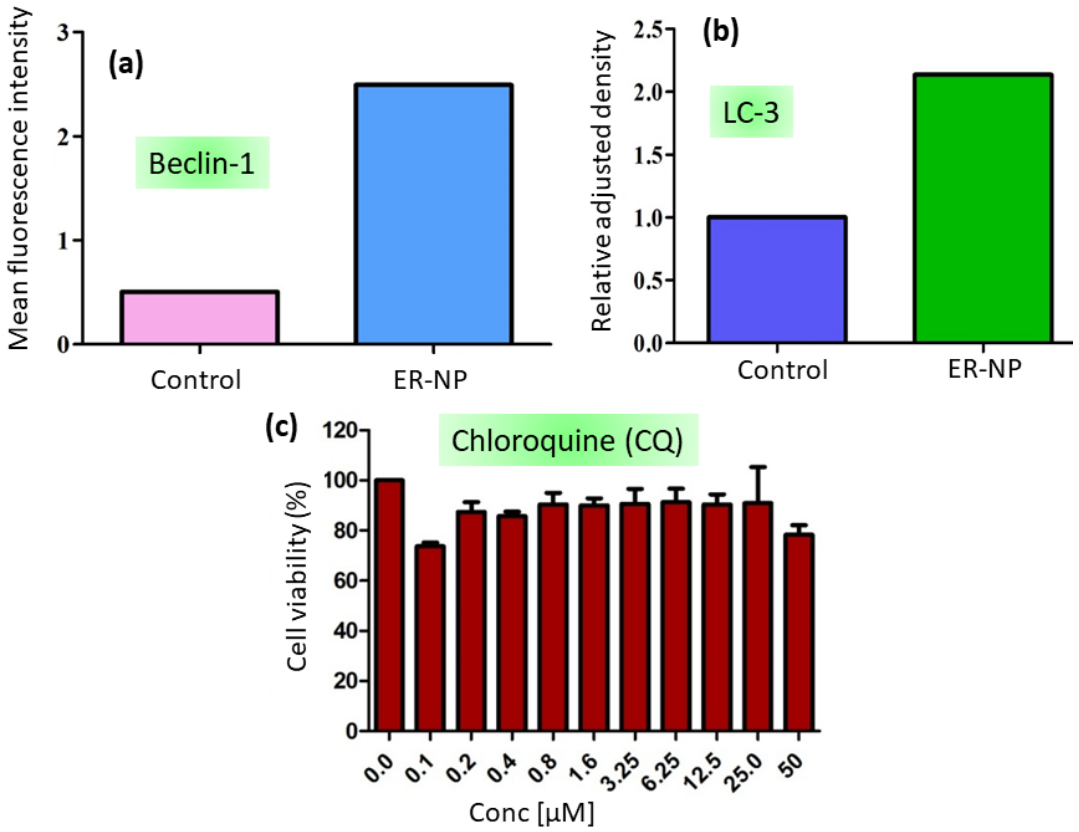

Fig. S28: (a) Quantification of Beclin-1 expression from CLSM images in HeLa cells after treatment with ER-NPs for 24 h. (b) Quantification of expression of LC-3 from Western blot in HeLa cells after treatment with ER-NPs for 24 h. (c) Cell viability of HeLa cells after treatment with chloroquine (CQ) at 48 h post-incubation.

		1h	6h	24h
Image Channels		C2 (green) C3 (red)	C2 (green) C3 (red)	C2 (green) C3 (red)
Pearsons' Correlation Coefficient	R	0.8821	0.8795	0.8324
Manders Coefficients	M1 (fraction of C2 overlapping C3)	0.8971	0.8913	0.8309
	M2 (fraction of C3 overlapping C2)	0.8892	0.9031	0.8458
Percent volume Co-localized		***55.23%***	***56.11%***	***44.24%***

Table S1: Quantification of co-localization of compound **11** in ER of HeLa cells at 1 h, 6 h and 24 h from CLSM.

		30mins	1h	6h
Image Channels		C2 (green) C3 (red)	C2 (green) C3 (red)	C2 (green) C3 (red)
Pearsons' Correlation Coefficient	R	0.9421	0.7995	0.9724
Manders Coefficients	M1 (fraction of C2 overlapping C3)	0.9462	0.9387	0.9204
	M2 (fraction of C3 overlapping C2)	0.9489	0.9515	0.9302
Percent volume Co-localized		***68.98%***	***60.13%***	***52.24%***

Table S2: Quantification of co-localization of ER-NPs in ER of HeLa cells at 30 min, 1 h and 6 h from CLSM.

		Control	Genistein	Chlorpromazine	Amiloride
Image Channels		C2 (green) C3 (red)	C2 (green) C3 (red)	C2 (green) C3 (red)	C2 (green) C3 (red)
Pearsons' Correlation Coefficient	R	0.8121	0.8723	0.8032	0.4356
Manders Coefficients	M1 (fraction of C2 overlapping C3)	0.9588	0.9452	0.9750	0.7645
	M2 (fraction of C3 overlapping C2)	0.9880	0.9650	0.9130	0.7820
Percent volume colocalized		***49.87%***	***54.21%***	***44.61%***	***22.07%***

Table S3: Quantification of co-localization of ER-NPs into ER in HeLa cells pre-treated with different endocytosis inhibitors from CLSM.

		24h	24h (Zoom)
Image Channels		C2 (green) C3 (red)	C2 (green) C3 (red)
Pearsons' Correlation Coefficient	R	0.7720	0.7890
Manders Coefficients	M1 (fraction of C2 overlapping C3)	0.8123	0.9041
	M2 (fraction of C3 overlapping C2)	0.8341	0.9205
Percent volume colocalized		***35.55%***	***39.21%***

Table S4: Quantification of co-localization of ER-NPs into mitochondria in HeLa cells at 24 h from CLSM.

		24h	24h (zoom)
Image Channels		C2 (green) C3 (red)	C2 (green) C3 (red)
Pearsons' Correlation Coefficient	R	0.2574	0.3028
Manders Coefficients	M1 (fraction of C2 overlapping C3)	0.8200	0.8541
	M2 (fraction of C3 overlapping C2)	0.8104	0.8622
Percent volume colocalized		***21.08%***	***16.56%***

Table S5: Quantification of co-localization of ER-NPs into lysosomes in HeLa cells at 24 h from CLSM.