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

Supporting Information

A facile method for synthesis of copper-cysteamine nanoparticles and study of ROS production for cancer treatment

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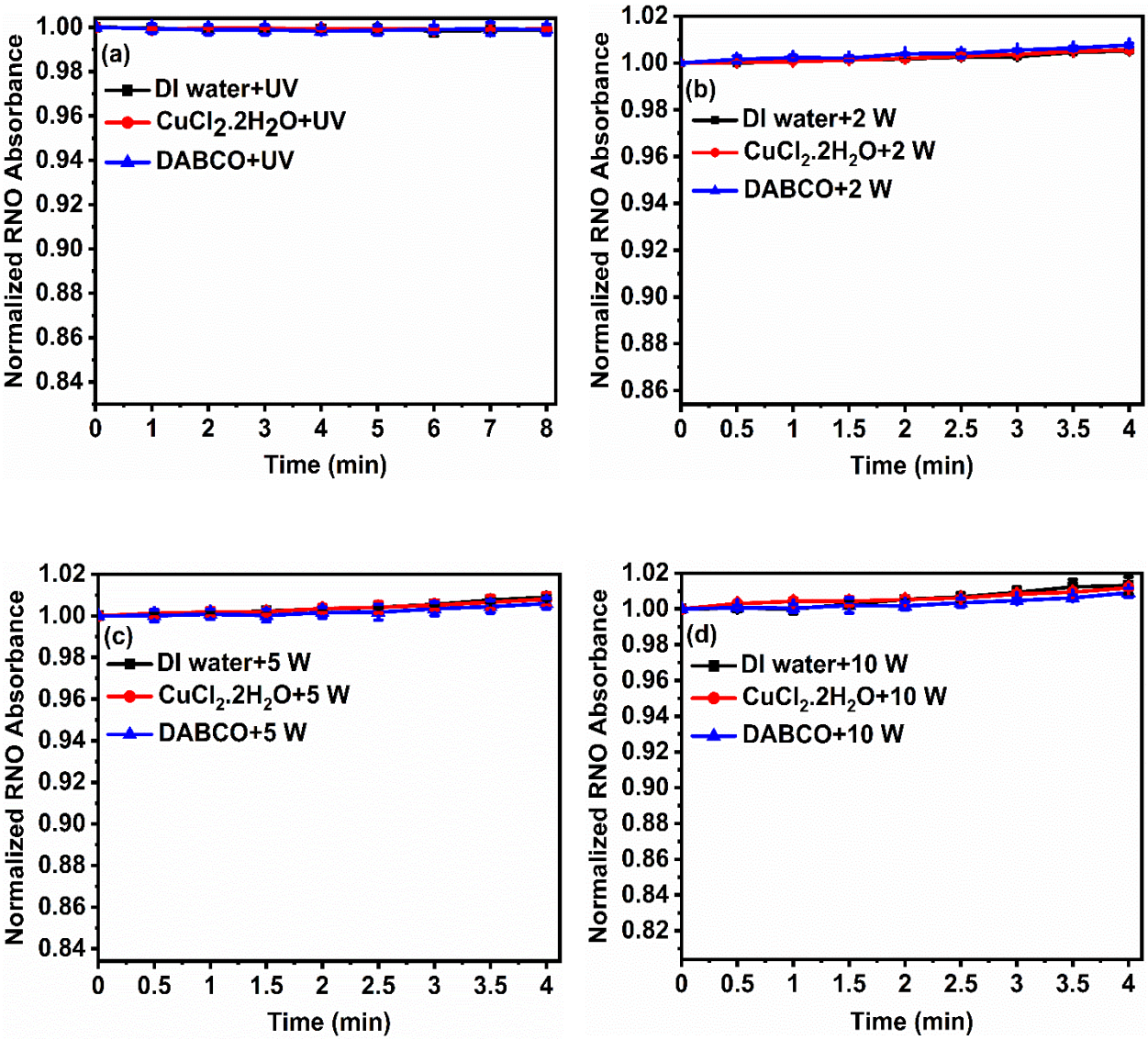

Fig. S1 Normalized RNO absorbance curves of DI water (control), $CuCl_2.2H_2O$ (933 µM), and DABCO (20 mM) at 440 nm under (a) UV light irradiation for 0-8 min in the interval of 1 min, (b) 2 W of MW irradiation for 0-4 min in the interval of 30 seconds, (c) 5 W of MW irradiation for 0-4 min in the interval of 30 seconds, and (d) 10 W of MW irradiation for 0-4 min in the interval of 30 seconds**.** These data show that ROS generated by $CuCl_2.2H_2O$ under our experimental conditions cannot be measured by RNO-ID method. Also, the above results display that DABCO alone does not have any effect on RNO absorbance. The data are expressed as the mean ± standard deviation of three independent experiments.

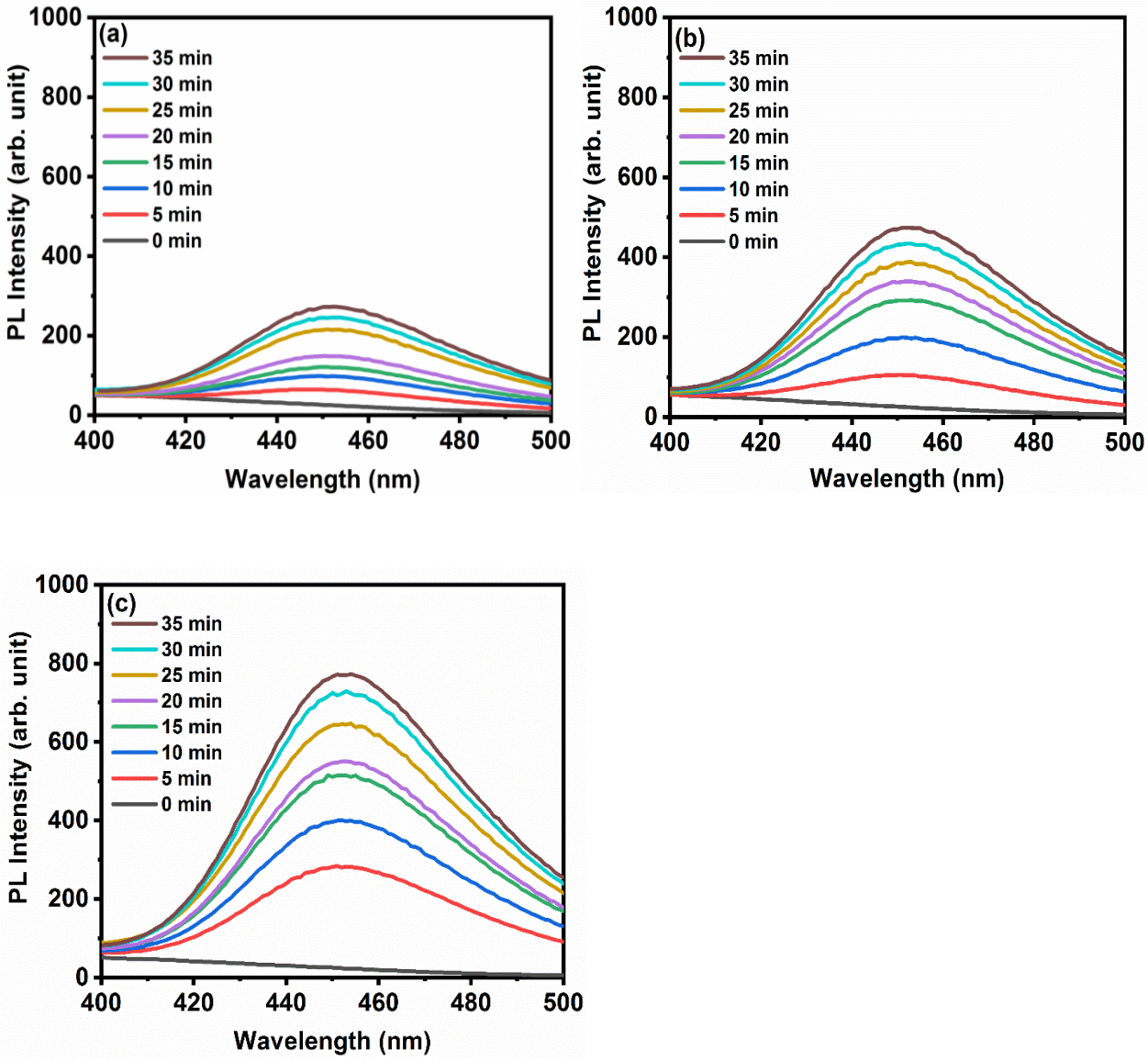

Fig. S2 Fluorescence spectra of 0.1 mM coumarin solutions of the first trial after exposing MW with (a) 2 W, (b) 5 W, and (c) 10 W for 0-35 min in the presence of Cu-Cy nanoparticles (35 µg/mL). The excitation wavelength was at 332 nm. Increase of the intensity while increasing MW power and time reflects the formation of 7-hydroxycoumarin.

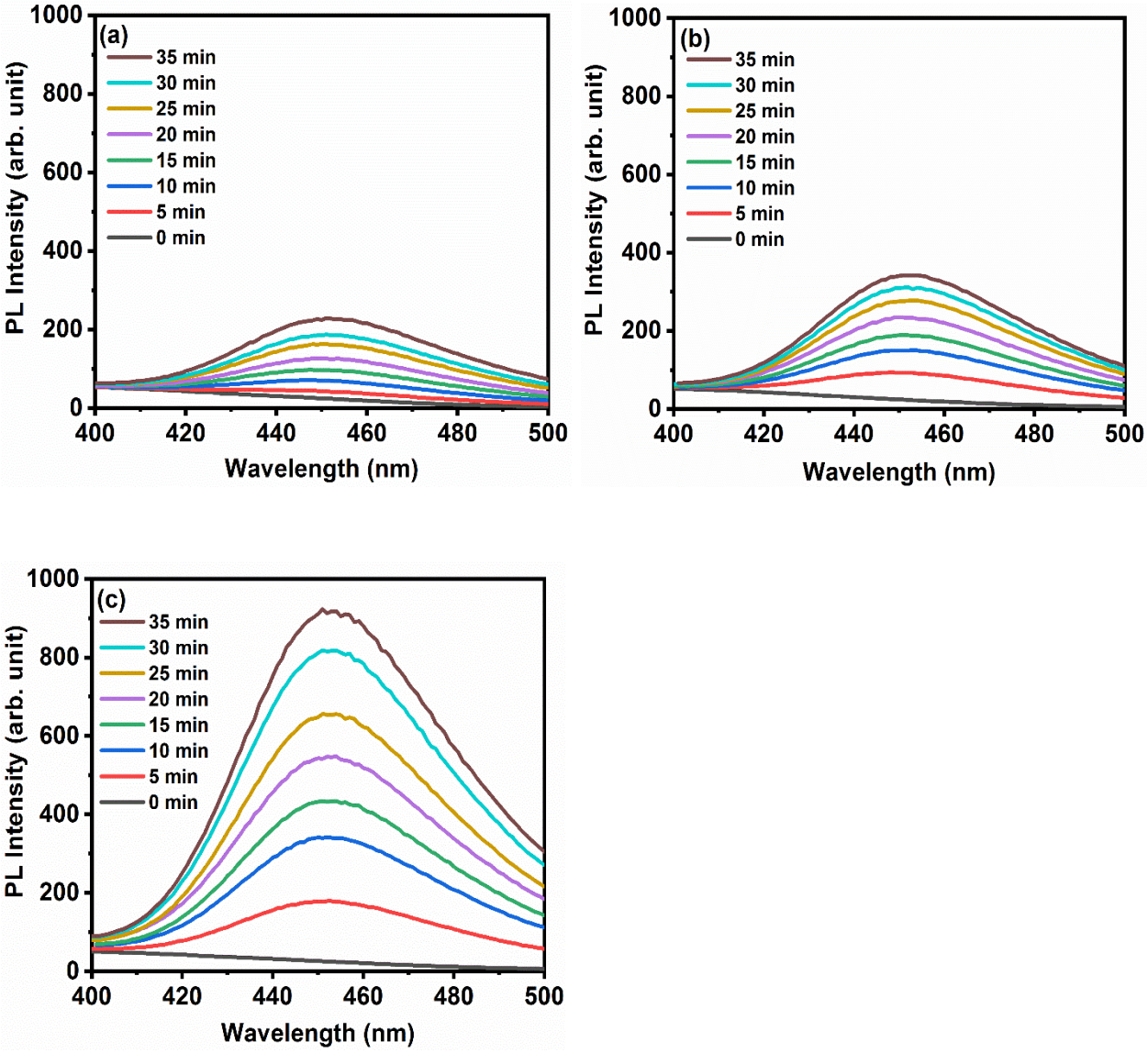

Fig. S3 Fluorescence spectra of 0.1 mM coumarin solutions of the second trial after exposing MW with (a) 2 W, (b) 5 W, and (c) 10 W for 0-35 min in the presence of Cu-Cy nanoparticles (35 µg/mL). The excitation wavelength was at 332 nm.

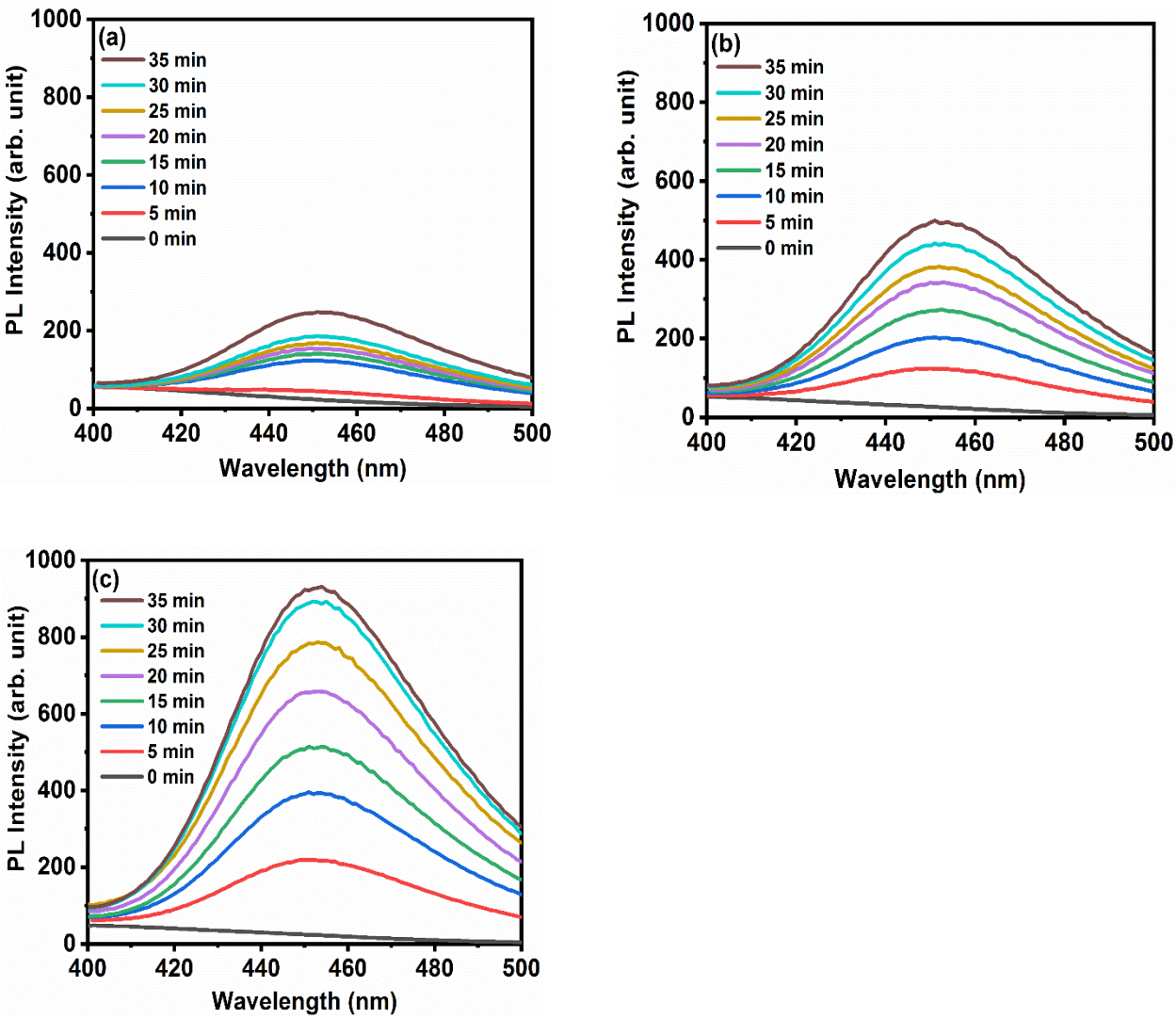

Fig. S4 Fluorescence spectra of 0.1 mM coumarin solutions of the third trial after exposing MW with (a) 2 W, (b) 5 W, and (c)10 W for 0-35 min in the presence of Cu-Cy nanoparticles (35 µg/mL). The excitation wavelength was at 332 nm.

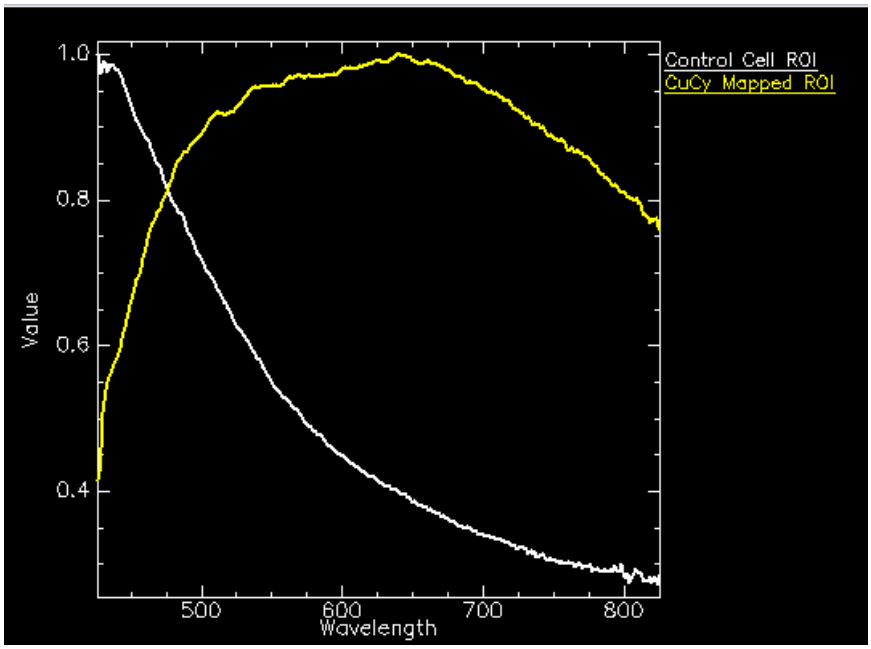

Fig. S5 Comparison of reflectance spectra of the cells with and without the Cu-Cy nanoparticles.

Cell Apoptosis Study by Hoechst 33342 Staining

Apoptosis and necrosis are two major pathways of cell death. [1] Changes in nuclear morphology, chromatin condensation, and nuclei fragmentation are the main indicators of cellular apoptosis. [1-5] In order to explore whether apoptosis is one of the causes of cell death upon MW exposure after using Cu-Cy nanoparticles, Hoechst 33342 staining was adopted.

For this study, KYSE-30 cells were seeded into a 6-well plate (1×10^5 cells/well) for 24 h at 37 °C in a humidified atmosphere of 5 % CO_2 in a cell incubator. The cells were divided into four groups: control, MW, Cu-Cy, and Cu-Cy + MW. After 24 h of seeding, the old media was removed. 2 mL fresh media was separately added to the control and MW groups, and 2 mL media containing 7.5 µg Cu-Cy nanoparticles (3.75 µg/mL) was added to the Cu-Cy and Cu-Cy + MW groups. After 6 h, 30 seconds of MW (5 W) was applied to the MW and Cu-Cy + MW groups. The cells were further incubated for 20 h. Following the incubation, the old media was removed, the cells were washed 3 times with PBS and fixed with PFA for 15 min. Then, Hoechst

33342 dye was added to stain nucleus for 15 min at room temperature. The cells were subsequently observed under an OLYMPUS IX71 fluorescence microscope to study the cellular apoptosis.

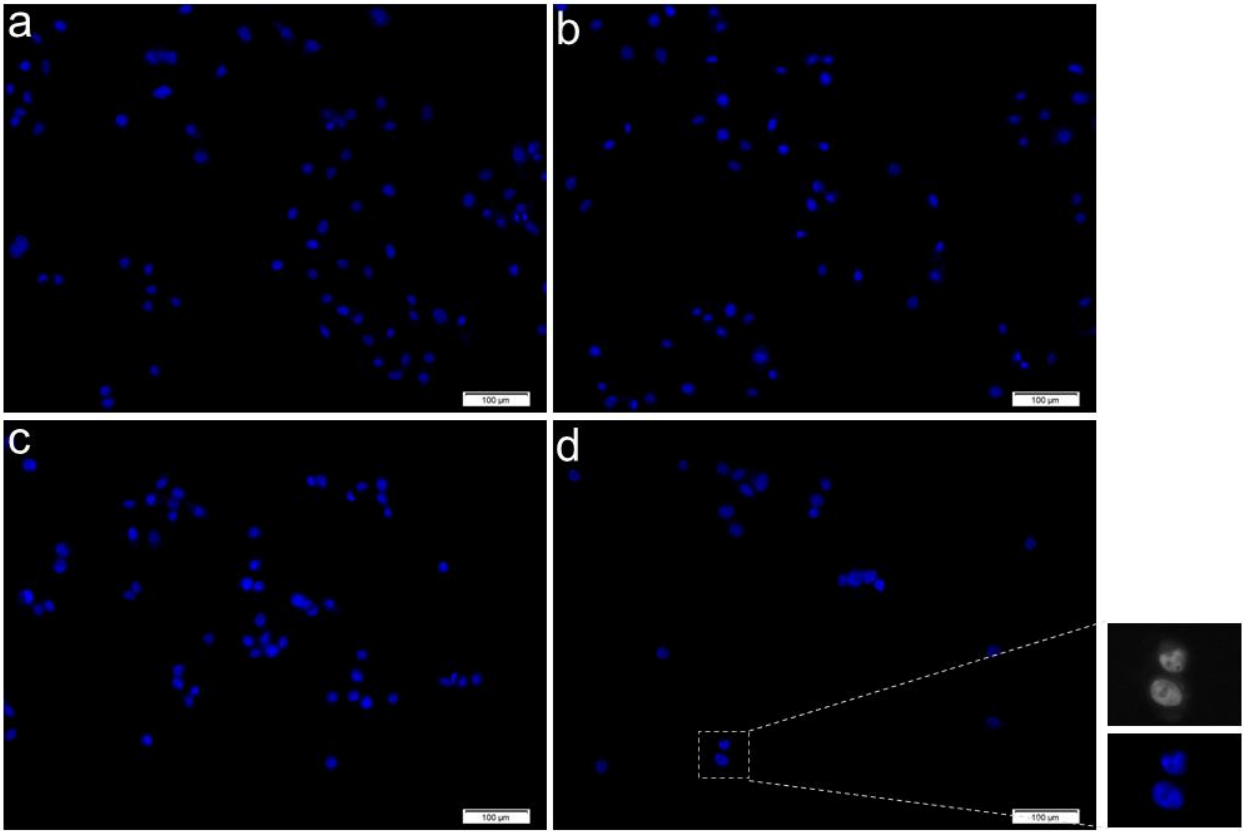

Fig. S6 The Effect of Cu-Cy nanoparticles with MW treatment on the induction of apoptosis in KYSE-30 cancer cells. (a) Control group, (b) MW (30 seconds, 5 W), (c) Cu-Cy nanoparticles (3.75 μg/mL), and (d) Cu-Cy nanoparticles (3.75 μg/mL) + MW (30 seconds, 5 W). Scale bar: 100 μm

The result showed in Fig. S6 shows that MW or Cu-Cy nanoparticles alone could not induce apoptosis. However, when Cu-Cy was stimulated by MW, we could observe the cell shrinkage. Also, the nucleus became smaller and split into smaller apoptotic bodies. It indicates that one of the causes of cell death under MW stimulation is apoptosis.

References

1. T. Bezabeh, M. Mowat, L. Jarolim, A. Greenberg and I. Smith, *Cell death and differentiation*, 2001, **8**, 219.
2. S. Mukhopadhyay, R. S. Singh, R. P. Paitandi, G. Sharma, B. Koch and D. S. Pandey, *Dalton Transactions*, 2017, **46**, 8572-8585.
3. M. Ganeshpandian, R. Loganathan, E. Suresh, A. Riyasdeen, M. A. Akbarsha and M. Palaniandavar, *Dalton Transactions*, 2014, **43**, 1203-1219.
4. R. P. Paitandi, S. Mukhopadhyay, R. S. Singh, V. Sharma, S. M. Mobin and D. S. Pandey, *Inorganic chemistry*, 2017, **56**, 12232-12247.
5. G. Majno and I. Joris, *The American journal of pathology*, 1995, **146**, 3.