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

Supporting Information

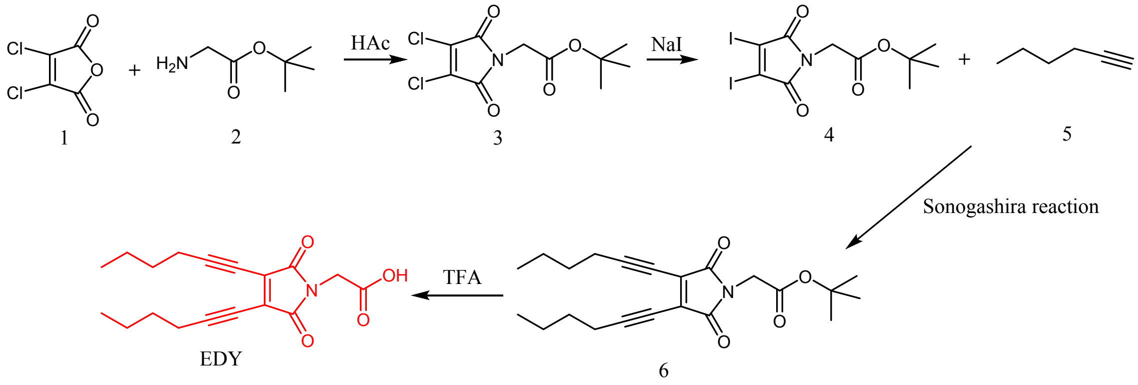
Self-delivery nanoparticles of amphiphilic irinotecan-enediyne conjugate for cancer combination chemotherapy

1. Materials

N,*N*'-Dicyclohexylcarbodiimide (DCC, 99%, J&K), 4-(dimethylamino)pyridine (DMAP, 99%, J&K), Nile red (NR, 98%, Spectrum Chemical), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma), and Irinotecan (Ir) was purchased from Shanghai Energy Chemical company. Alexa fluorR 488 annexin V/dead cell apoptosis assay kit was purchased from Invitrogen and used as received. Chloroform ($CHCl_3$) was dried over calcium hydride and distilled before use. All other reagents and solvents were purchased from the domestic suppliers and used as received.

2. Characterizations

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using a Ultra Shield 500 spectrometer (BRUKER BIOSPIN AG, Magnet System 500 MHz/54 mm) and referenced to Me_4Si with deuterium chloroform ($CDCl_3$) as a solvent. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer 3,000 HS (Malvern Instruments, Ltd.) equipped with a 125 mW laser light and operated at λ = 633 nm. All samples were measured at a scattering angle of 90°, and the data were the mean value of three tests. Mass spectra were recorded on a Micromass LCTTM mass spectrometer using the ESI method. Transmission electron microscopy (TEM) studies were performed with a JEOL JEM-100CX-II instrument at a voltage of 200 kV for the morphology observation. Samples were prepared by drop-casting nanoparticle solutions (0.1 mg mL^{-1}) onto carbon-coated copper grids and then were air-dried at room temperature before measurements. Fluorescent spectra were recorded on QC-4-CW spectrometer. The excitation wavelength was set at 360 nm, which was chosen according to the maximum intensity obtained in the excitation spectra. Electron spin resonance (ESR) spectroscopy studies were carried out with the enediynes dissolved in DMSO alone (40 mg mL^{-1}) or enediynes (40 mg mL^{-1}) and PBN (over-dose) dissolved in DMSO, and were measured with Bruker EMX-8/2.7C ESR spectrometer. The spectrometer settings were: sweep width, 100 G; time constant, 163.84 ms; conversion time, 40.96 ms; resolution, 1024 points; modulation amplitude, 1.00 G; and microwave power, 6.358 mW. UV-vis absorption spectra were recorded on a Shimadzu UV-2550 UV-vis spectrometer in methanol at room temperature.



Scheme S1.Synthesis of enediyne compound (EDY)

tert-butyl-2-(3,4-dichloro-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (Compound 3)[1]. Compound **1** (1.11 g, 10.1 mmol) was dissolved in acetic acid (20 mL) with slowly addition of tert-butyl glycinate (0.57 g, 10 mmol) solution in acetic acid (10 mL) drop by drop, then the solution was heated at 40 °C for 27 h. After removal of solvent, the crude residual was separated by column chromatography on silica gel (hexane/ethyl acetate 15/1) to give compound **3** (1.5 g, 52 %).

tert-butyl 2-(3,4-diiodo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (Compound 4) . A solution of compound **3** (1.5 g, 5.36 mmol) and sodium iodide (3.2 g, 21.43 mmol) in acetonitrile (40 mL) was heated and refluxed at 90 °C for 6 h. Then the solution was added to water with yellow floccules precipitated. The product was obtained after filtration, washing with water and drying at 60 °C (2.2 g, 88.7 %).

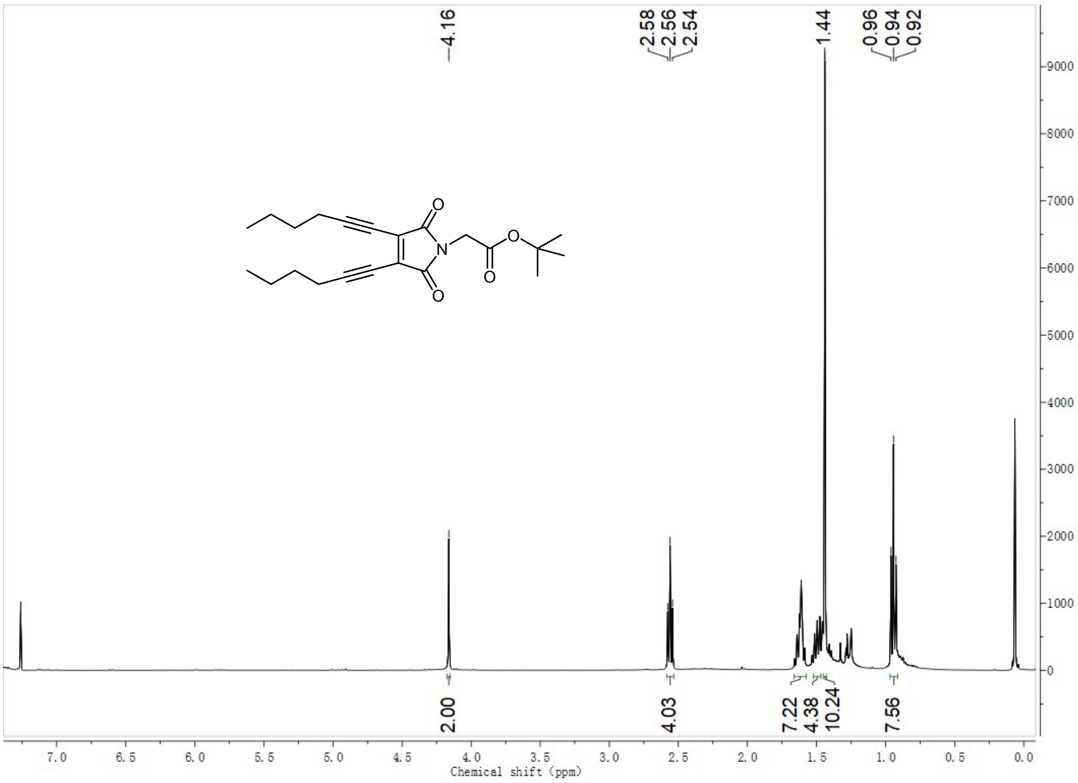


Fig S1. ^{1}H NMR spectrum of compound **6**

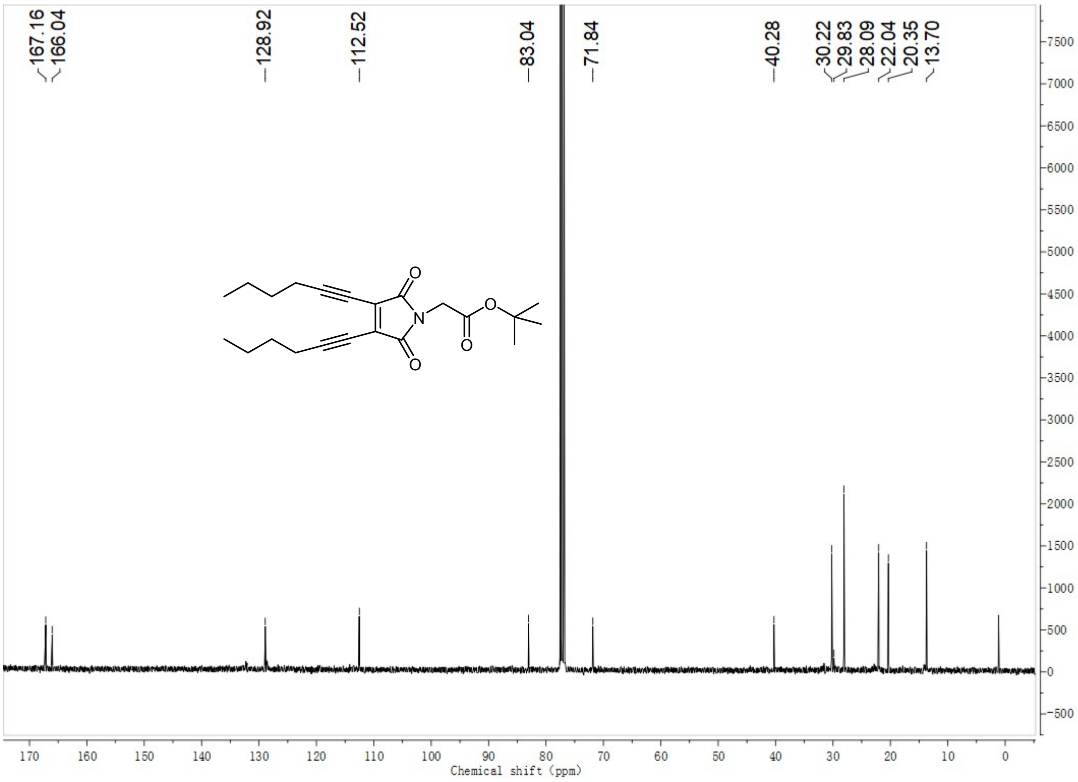


Fig S2. ^{13}C NMR spectrum of compound **6**

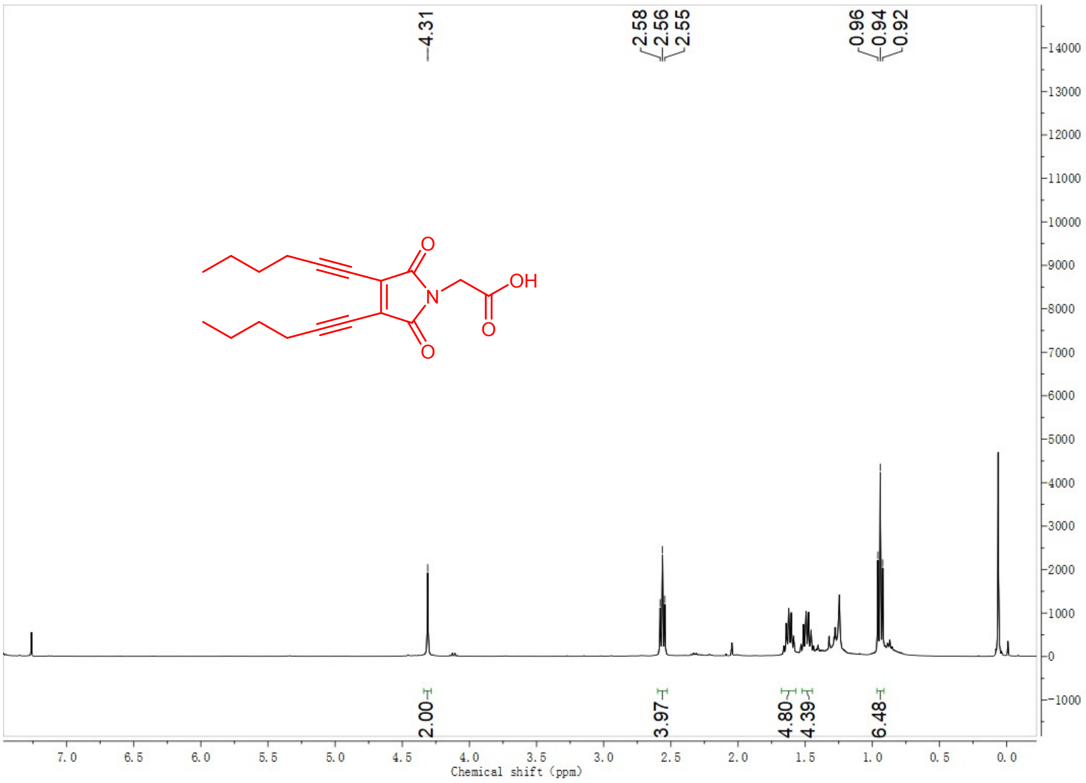


Fig S3. ^{1}H NMR spectrum of EDY

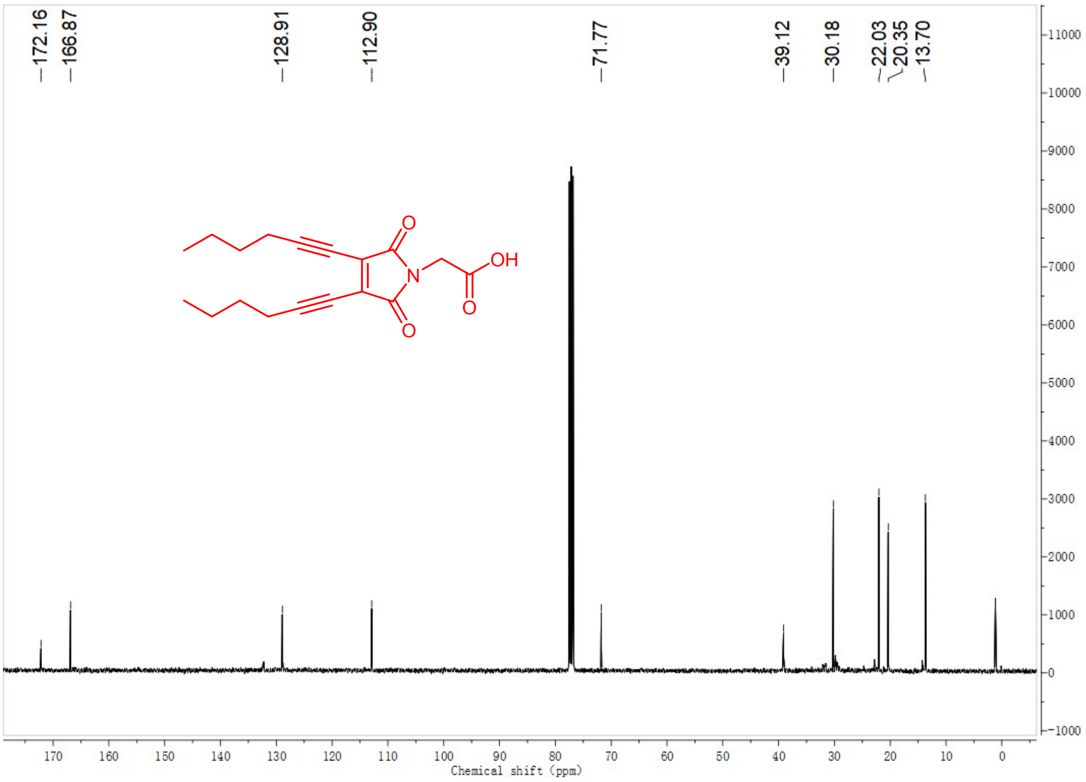


Fig S4. ^{13}C NMR spectrum of EDY

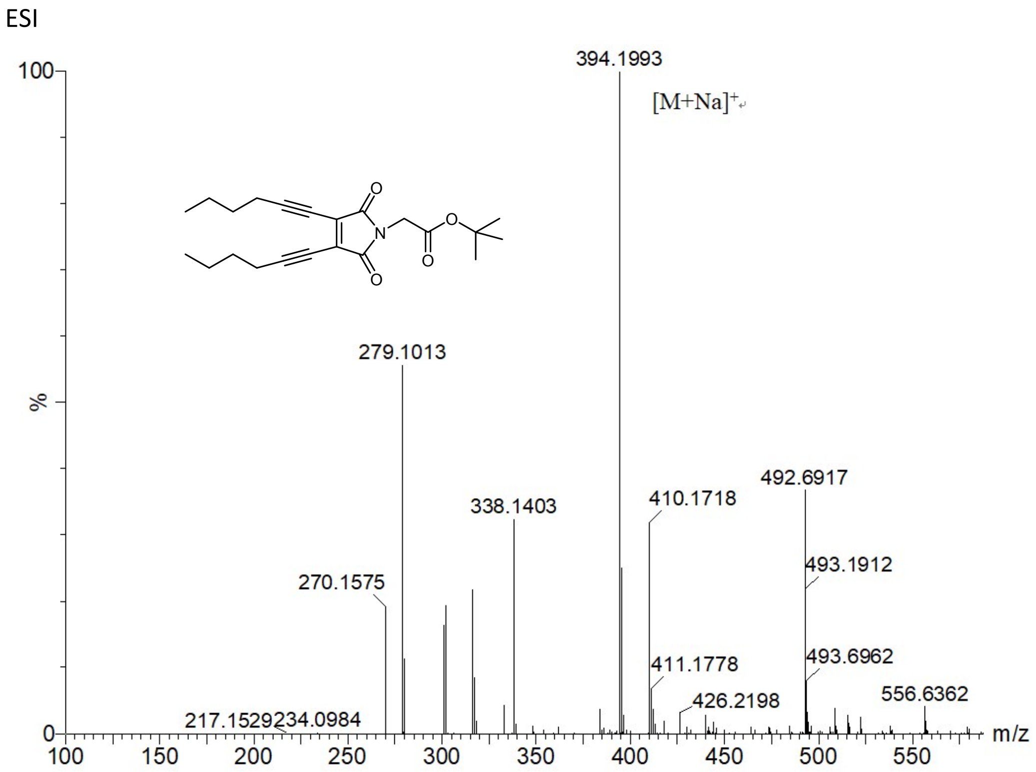


Fig S5. HR-MS spectrum of Compound 6

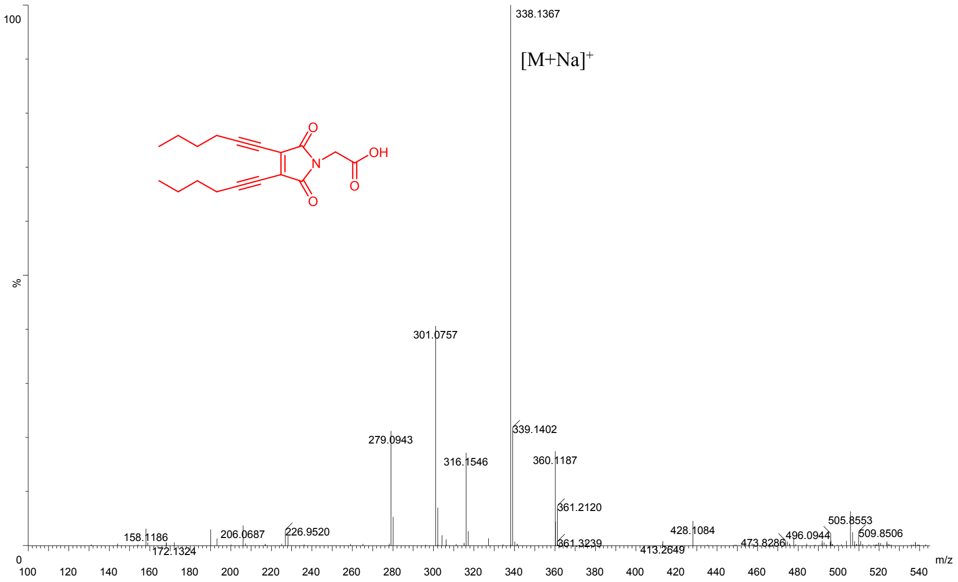


Fig S6. HR-MS spectrum of EDY

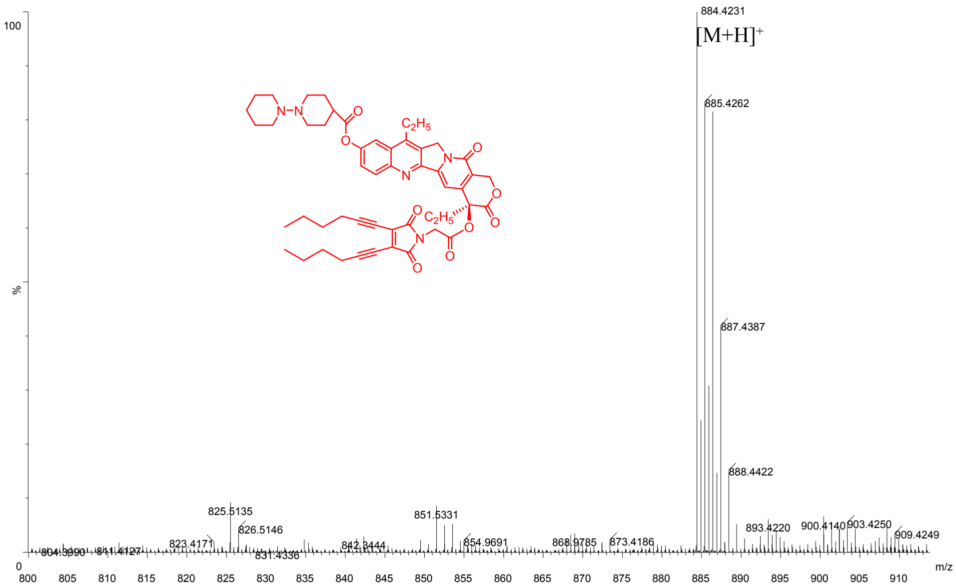


Fig S7. HR-MS spectrum of Ir-EDY

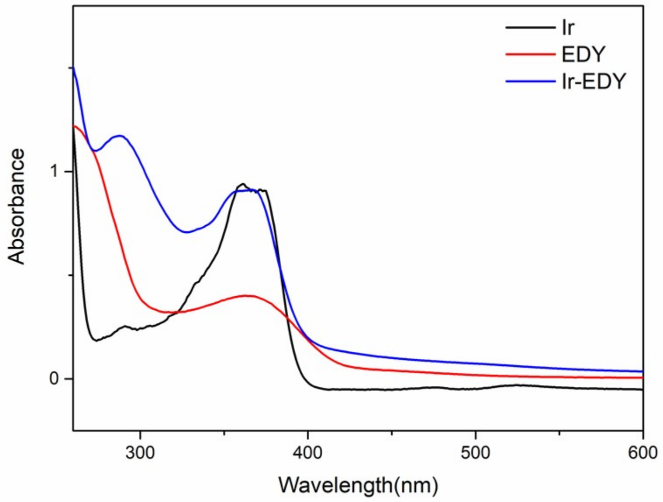


Figure S8. UV-Vis spectra of Ir, EDY, and Ir-EDY in DMSO.

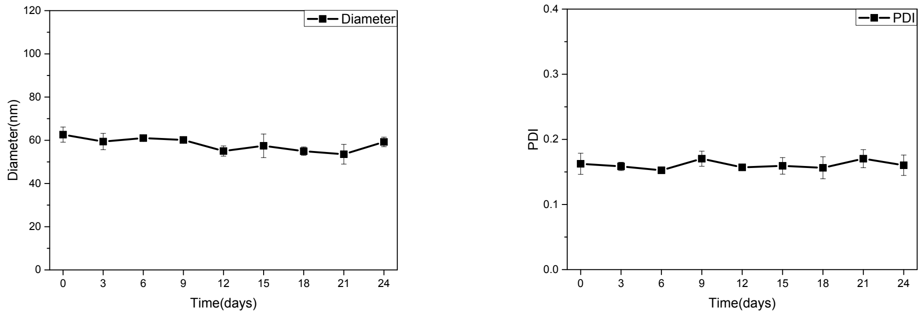


Figure S9. Influence of storage time on diameter (a) and PDI (b) of Ir-EDY ADDC nanoparticles. The solution of Ir-EDY nanoparticles was stored at 4 °C in a refrigerator for 24 days. At different time intervals (0, 3, 6, 9, 12, 15, 18, 21 and 24 d), the average size and PDI were determined. Samples were measured in triplicates. The values are the mean ± SD.

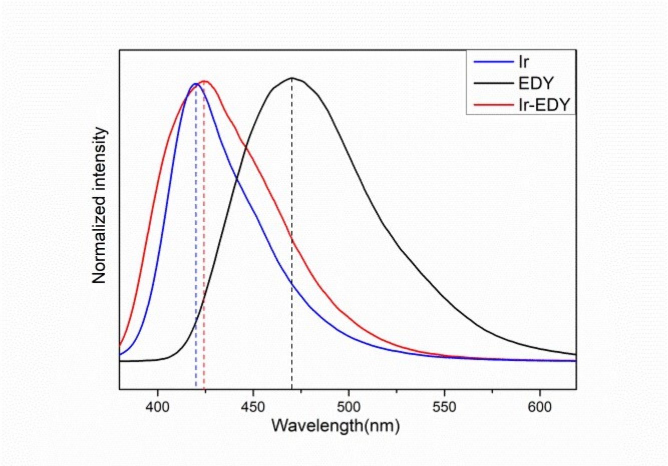


Figure S10. (**a**) Normalized fluorescence emission spectra of EDY in methanol ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 470$ nm), Ir ($\lambda_{ex} = 363$ nm, $\lambda_{em} = 420$ nm) and Ir-EDY conjugate ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 424$ nm) in aqueous solution.

Figure S11. Tyndall phenomenon of Ir-EDY NPs in aqueous solution.

Table S1. IC_{50} values calculated by Graphpad Prism

	Ir	EDY	Ir+EDY(mixture)	Ir-EDY NPs
IC_{50} (μM)	19.3	20.8	18	12.8

References

1. M. Dubernet, V. Caubert, J. Guillard and M. C. Viaud-Massuard, *Tetrahedron*, 2005, **61**, 4585-4593.