Supporting Information

Two-photon excited red-green "discoloration" bioprobes for monitoring lipid droplets and lipid droplet-lysosomal autophagy

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Content

S1. Synthetic route of DPABP-BI

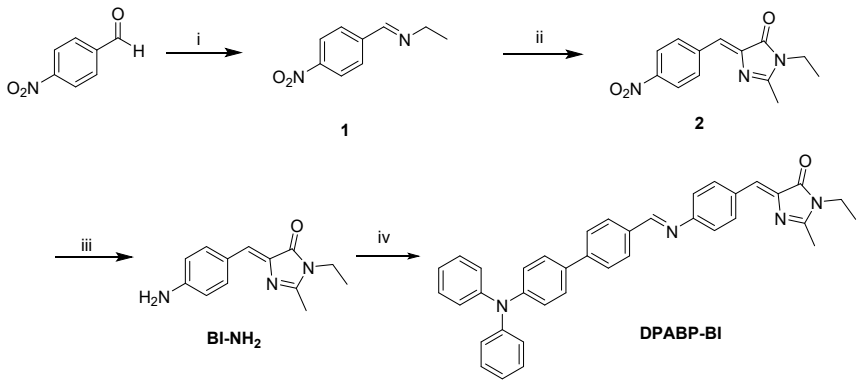

Fig. S1. Preparation of the compounds DPABP-BI: (i) ethylamine, ethanol, room temperature, 24 h; (ii) Methyl 2-((1-ethoxyethylidene)amino)acetate, room temperature, 24 h; (iii) Pd/C, H_2, room temperature, 30 minutes; (iv) tris(4-formylphenyl)amine, ethanol, room temperature, 48 h.

S2. The spectroscopic properties of DPABP-BI

Table S1 Photophysical data of **DPABP-BI**

solvent	λ_{ab} (nm)	λ_{em} (nm)	Stoke's shift (nm)	φ_F
DCM	485	610	125	0.23
THF	485	630	145	0.32
MeOH	485	600	115	0.18
DMF	493	600	107	0.30
DMSO	500	605	105	0.31
H_2O	490	605	115	0.08

Abbreviation: λ_{ab} = absorption maximum, λ_{em} = emission maximum, φ_F = fluorescence quantum yield.

S3. pH response of DPABP-BI

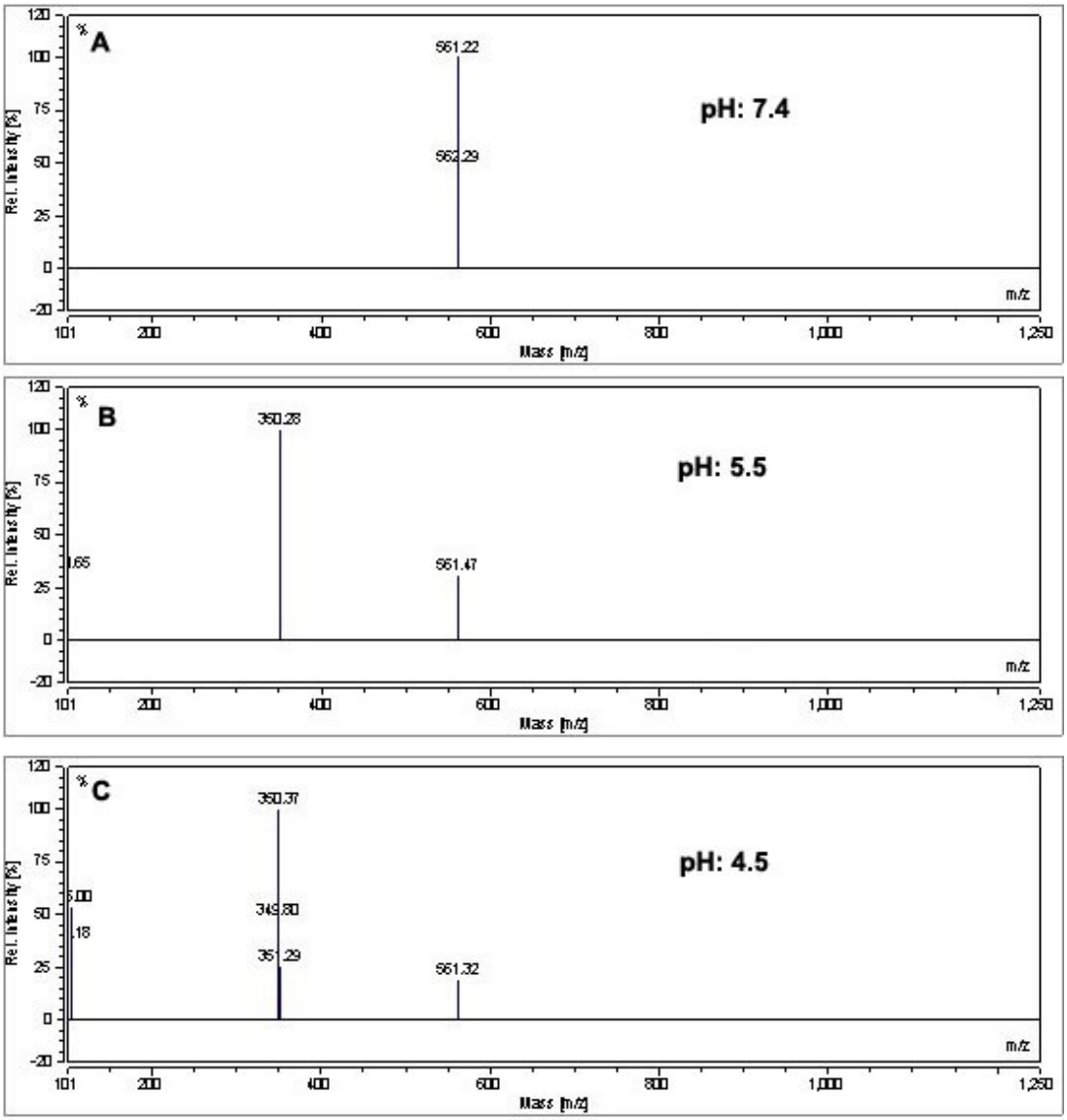

Fig. S2. MS spectrum of DPABP-BI at different pH.

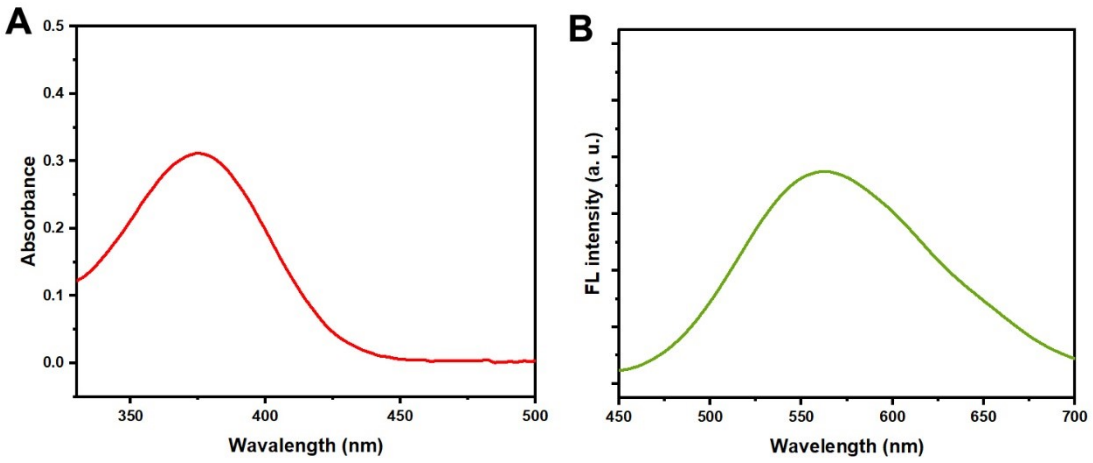

Fig. S3. (A) Absorption and (B) emission spectra of 4'-(diphenylamino)-[1,1'-biphenyl]-4-carbaldehyde in DMSO. Concentration: 10 μM.

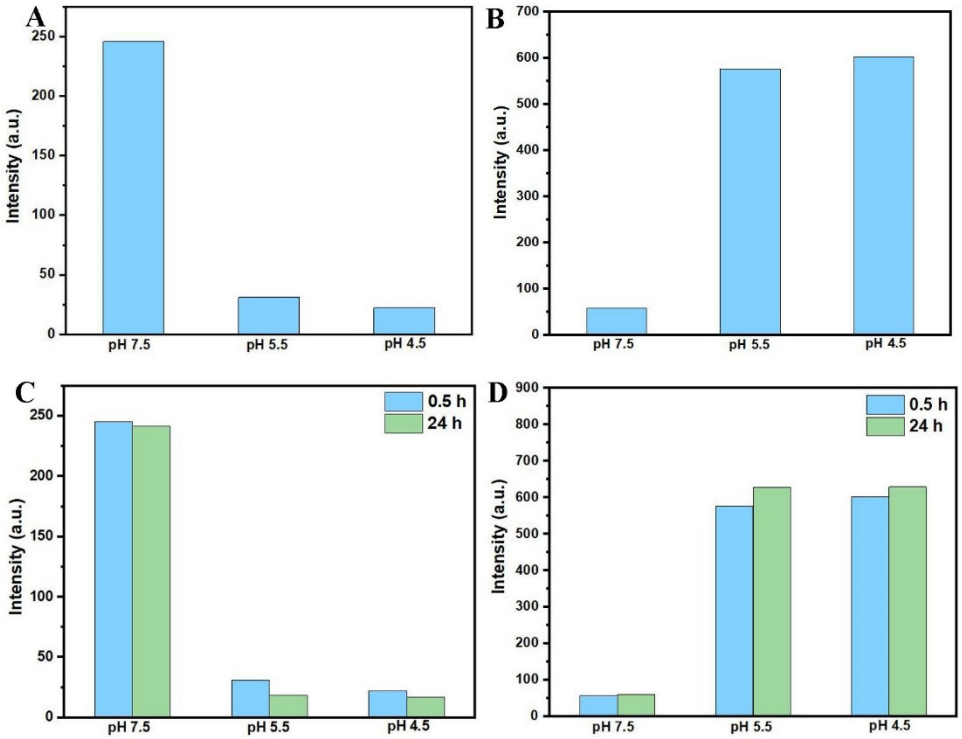

Fig. S4. The fluorescence of probe DPABP-BI at (A) 610 nm and (B) 550 nm at pH 7.5, 5.5 and 4.5, incubation time: 30 min. The fluorescence of DPABP-BI at (C) 610 nm and (D) 550 nm *vs* time at pH 7.5, 5.5 and 4.5. Concentration: 10 μM.

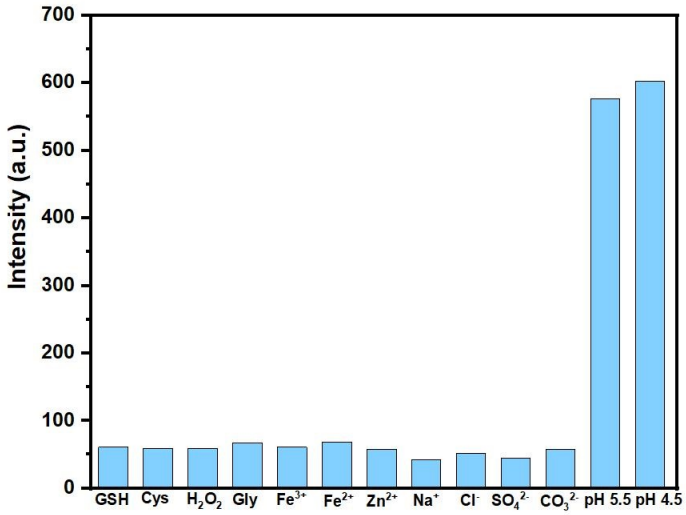

Fig. S5. Fluorescence intensity of DPABP-BI at 550 nm (the emission of the decomposed product 4'-(diphenylamino)-[1,1'-biphenyl]-4-carbaldehyde in the presence of different biologically active small molecules and ions at pH 7.5. Concentration: 10 μM (DMSO/Buffer = 1/1, v/v).

S4. Cytotoxicity test of DPABP-BI

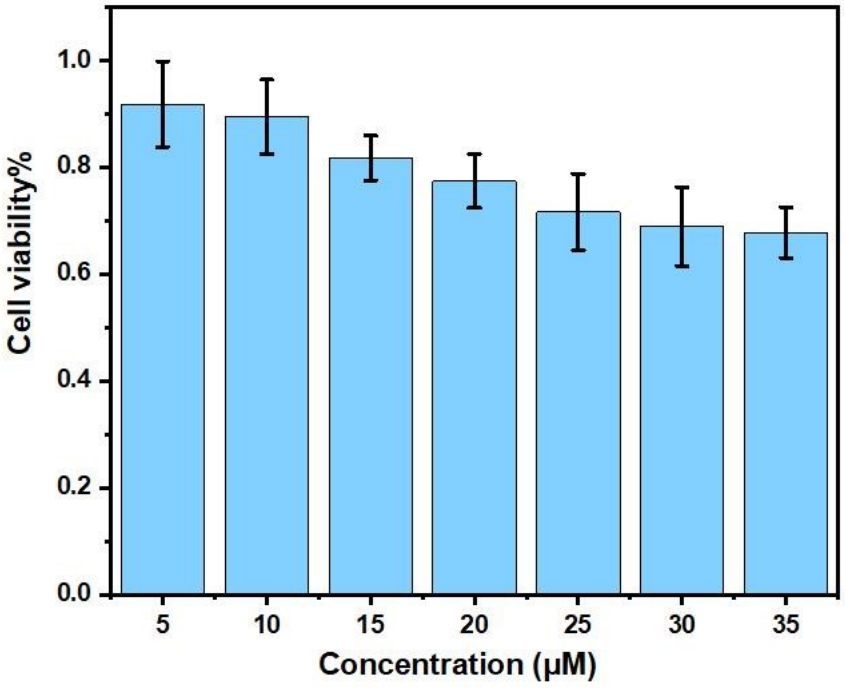

Fig. S6. Viability of HeLa cells incubated with DPABP-BI at different concentrations.

S5. Lipophilicity test of DPABP-BI

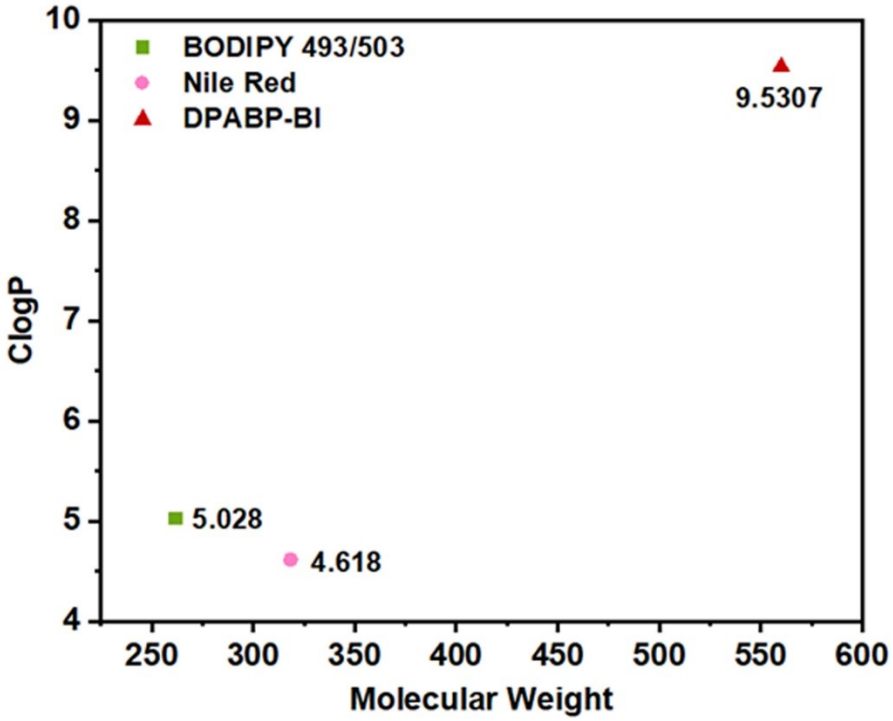

Fig. S7. ClogP values of BODIPY 493/503, Nile Red, and DPABP-BI.

S6. Study of autophagy processes

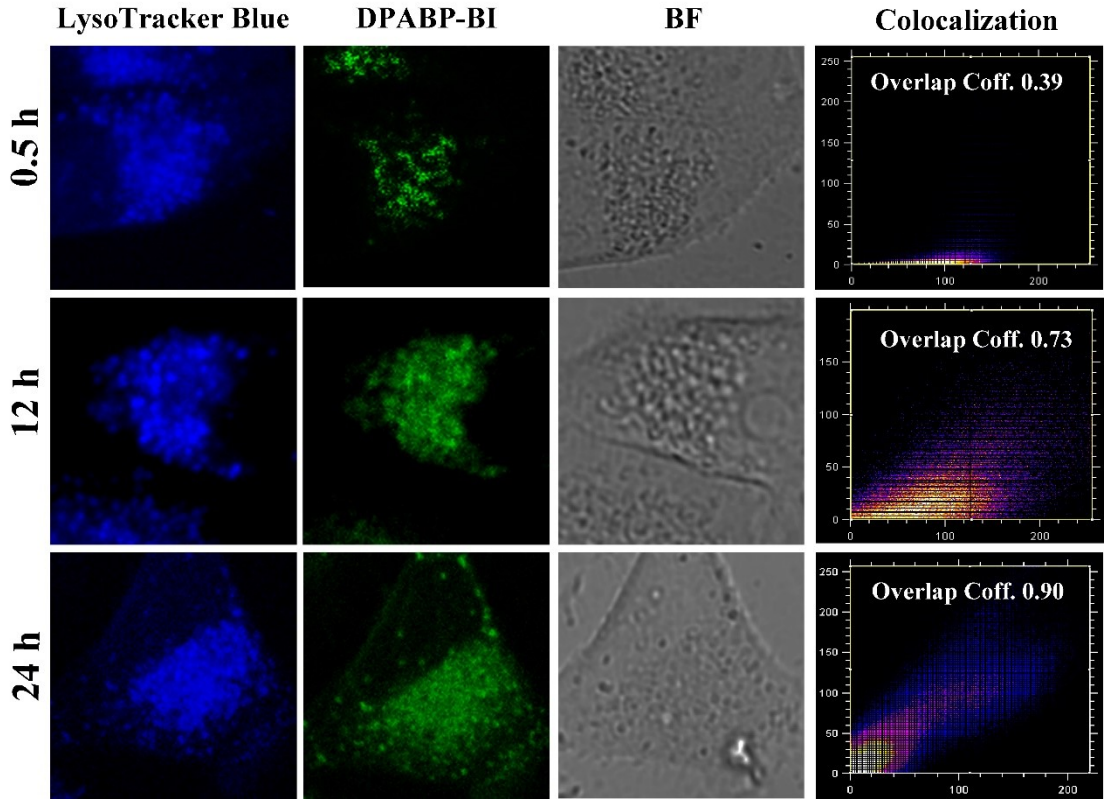

Fig. S8. CLMS images (400 X) of Hela cells incubated with 4 μM DPABP-BI (λ_{ex} = 380 nm and λ_{em} = 500–560 nm) and 4 μM LysoTracker Blue (λ_{ex} = 409 nm and λ_{em} = 425–475 nm) for different time.

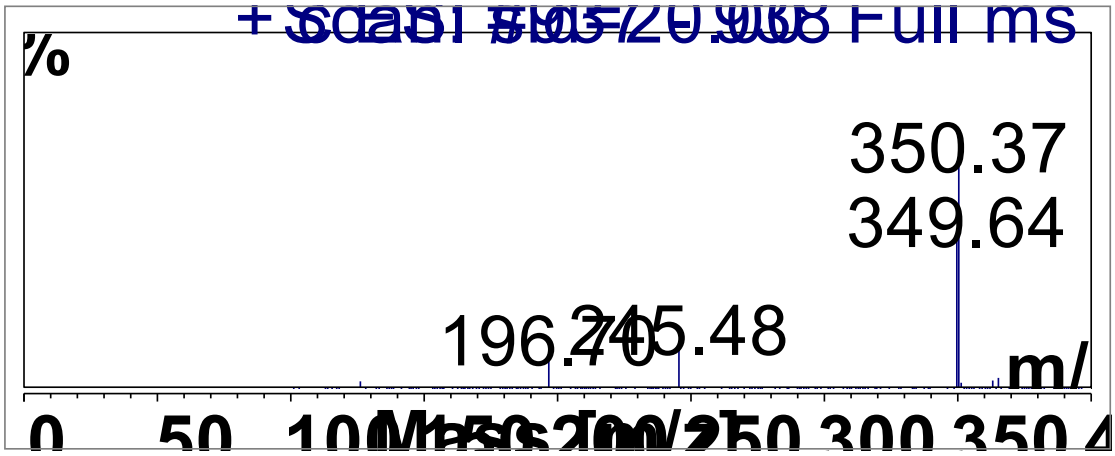

Fig. S9. Low-resolution mass spectrometry of DPABP-BI products during autophagy.

S7. Two-photon fluorescence test

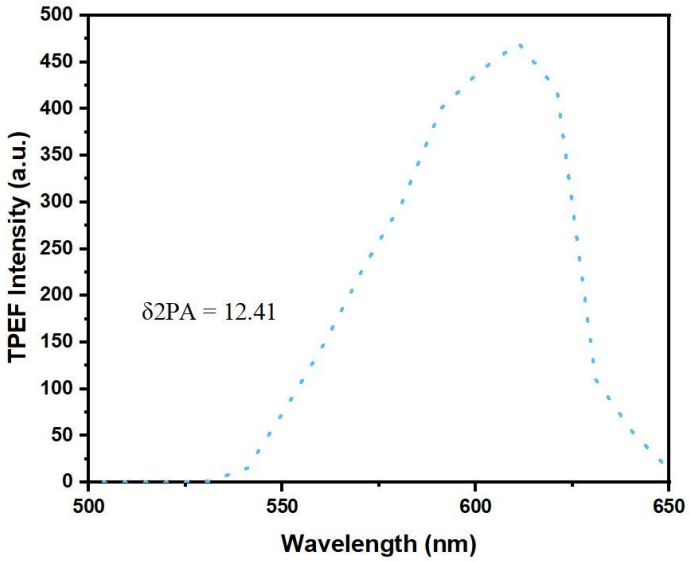

Fig. S10. Two-photon excited fluorescence spectra of DPABP-BI in THF**.** Concentration of DPABP-BI**:** 20 μM, excitation wavelength: 1000 nm.

S8. Two-photon imaging of DPABP-BI

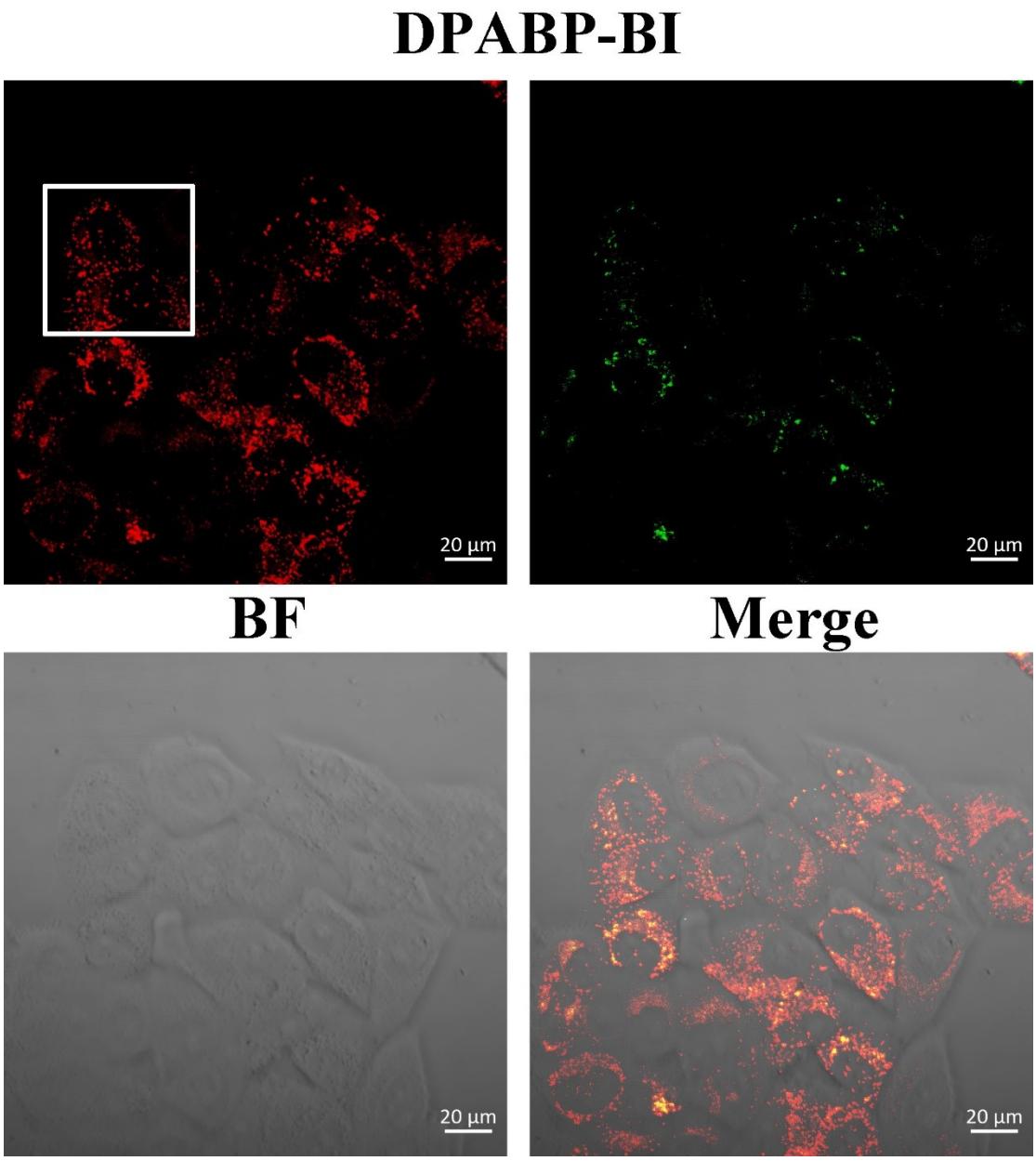

Fig. S11. CLMS images (400 X) of DPABP-BI in Hela cells. Incubation time: 30 min. Concentration: 4 μM, red channel: λ_{ex} = 1000 nm and λ_{em} = 600–720 nm, green channel: λ_{ex} = 760 nm and λ_{em} = 500–560 nm, Scale bar: 20 μm.

S9. ^{1}H-NMR and ^{13}C-NMR Spectra of compounds 1 to DPABP-BI

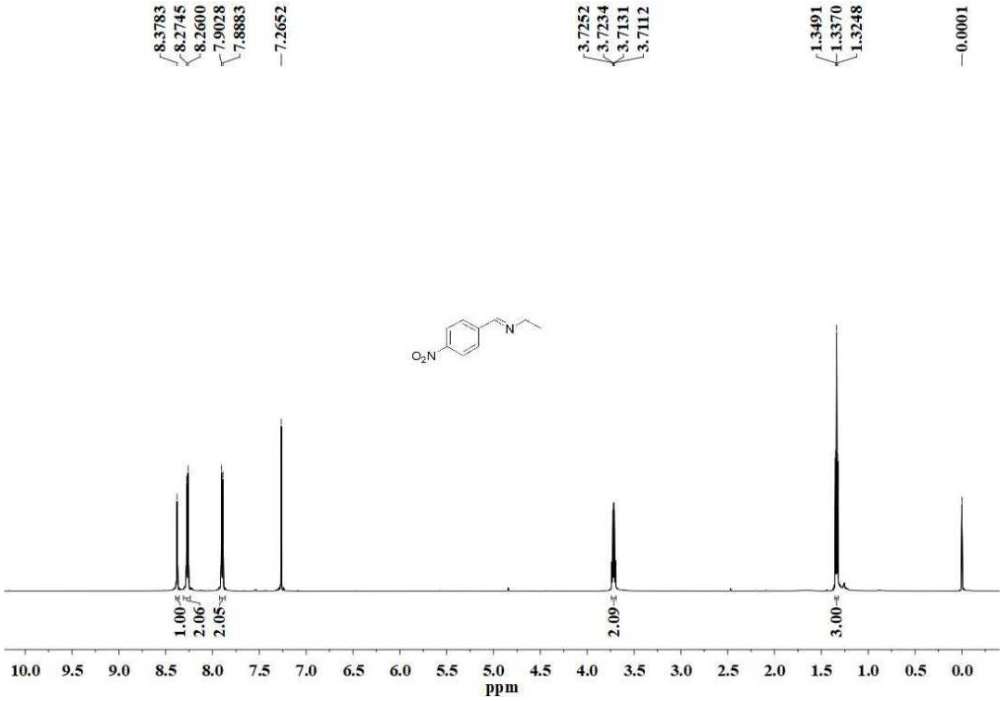

Fig. S12. ^{1}H NMR spectrum of **1** (600 MHz, $CDCl_3$)

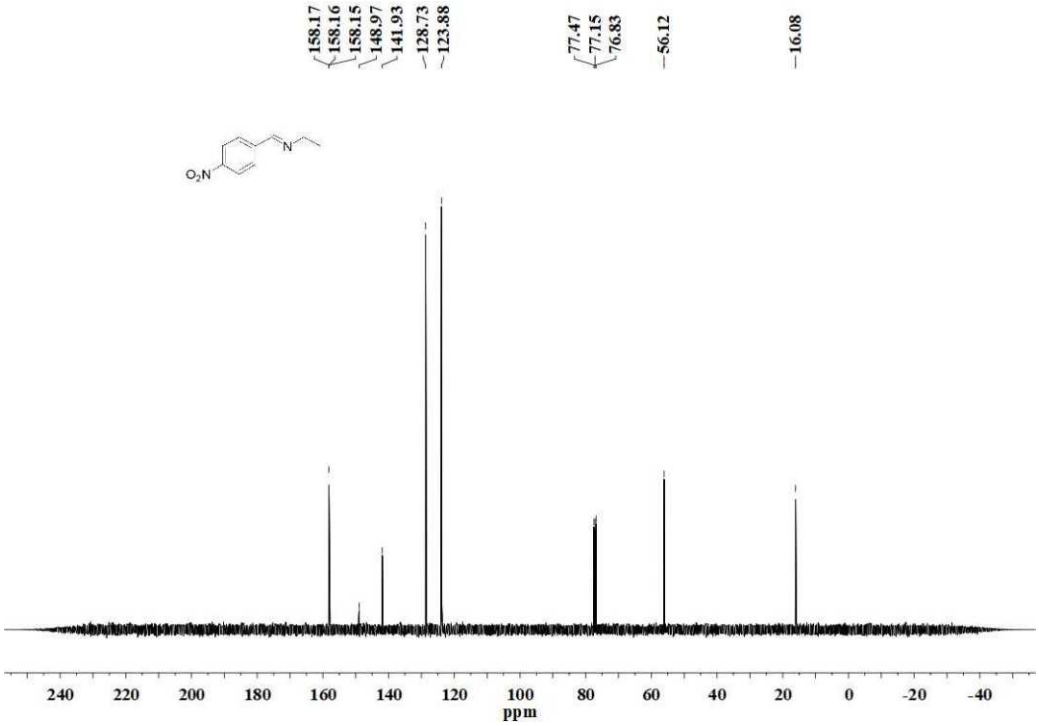

Fig. S13. ^{13}C NMR spectrum of **1** (101 MHz, $CDCl_3$)

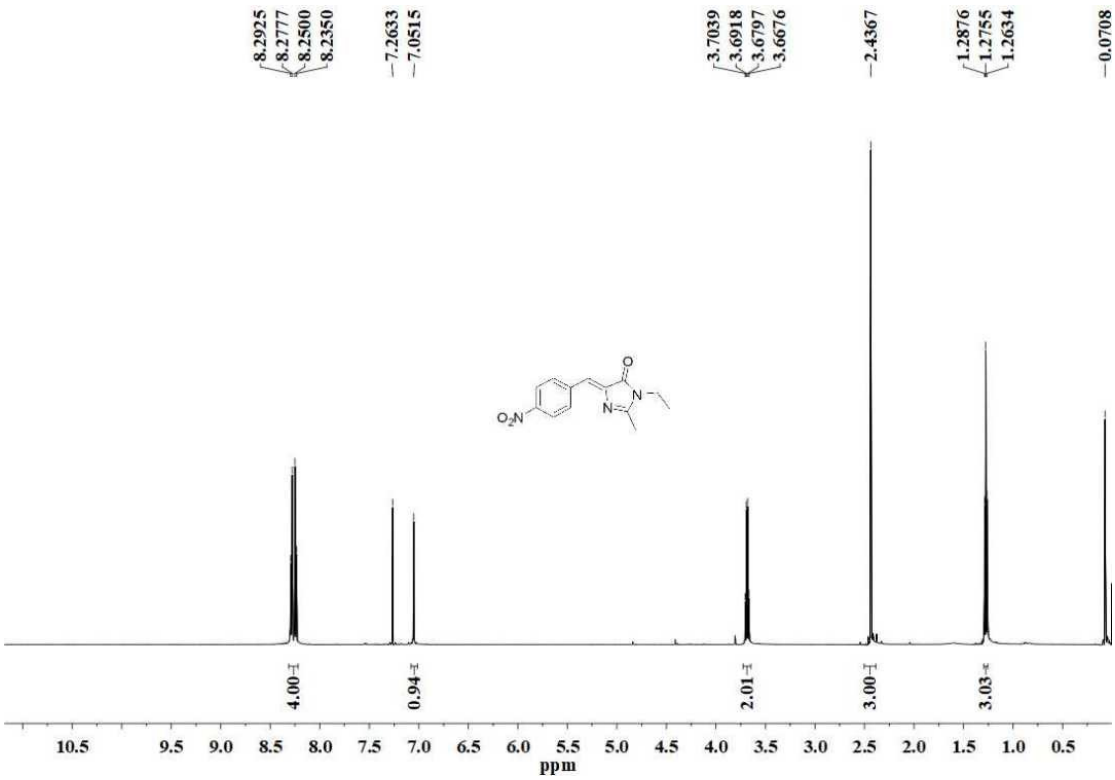

Fig. S14. ^{1}H NMR spectrum of **2** (600 MHz, $CDCl_3$)

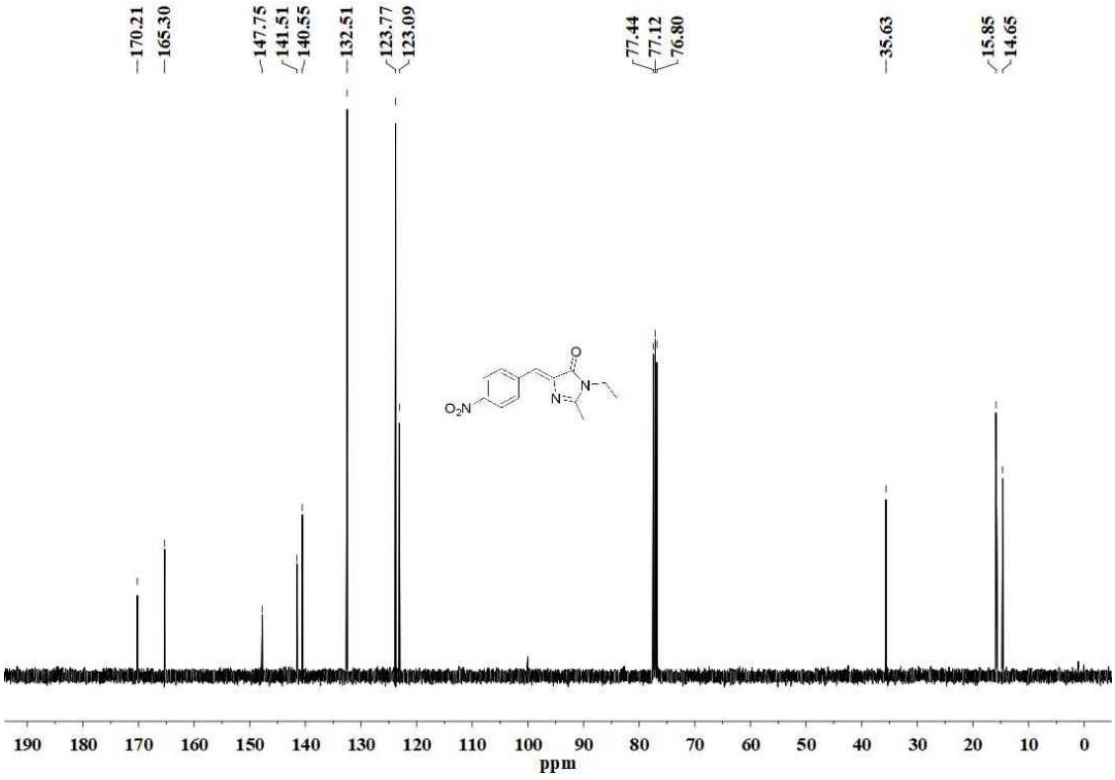

Fig. S15. ^{13}C NMR spectrum of **2** (101 MHz, $CDCl_3$)

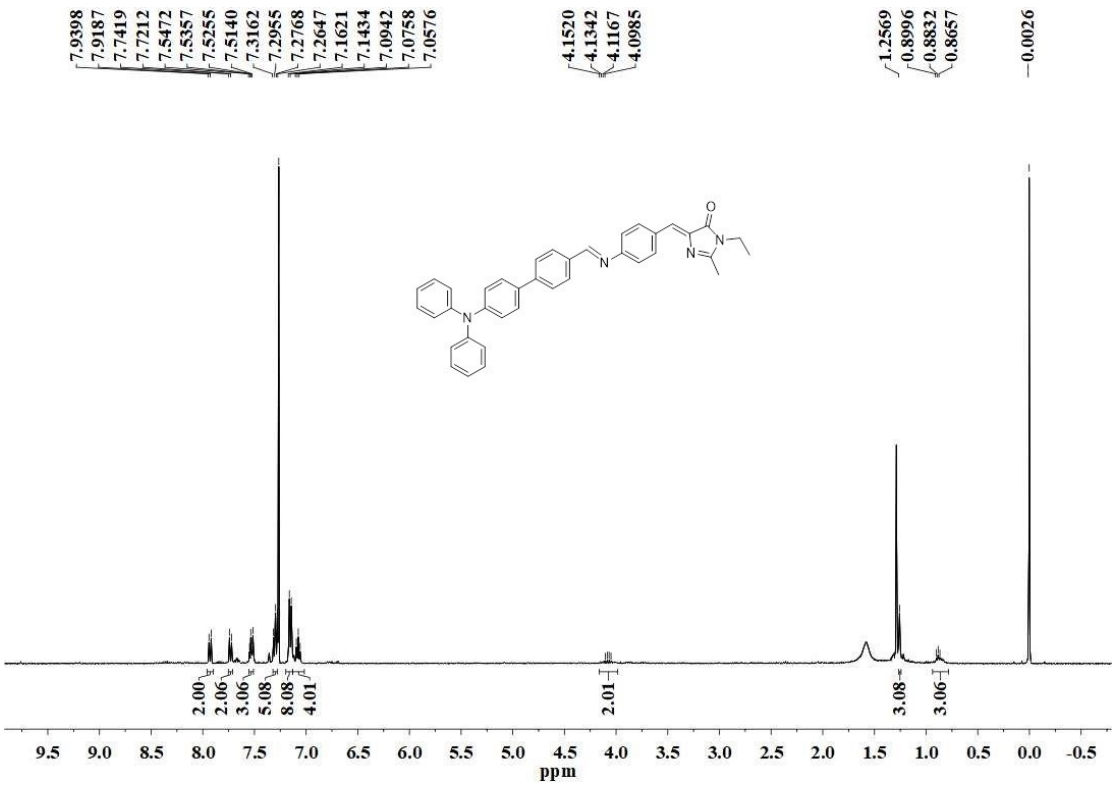

Fig. S16. [1]H NMR spectrum of DPABP-BI (400 MHz, $CDCl_3$)

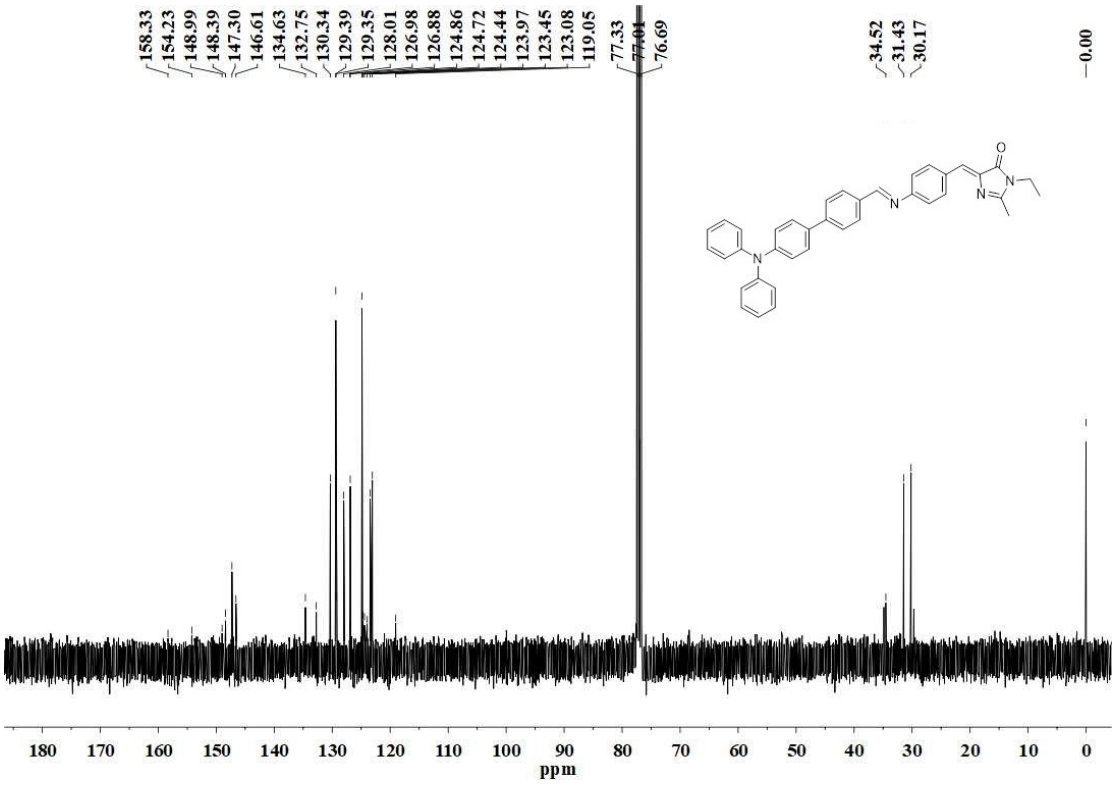

Fig. S17. [13]C NMR spectrum of DPABP-BI (101 MHz, $CDCl_3$)

S8. HR-MS Spectra of DPABP-BI

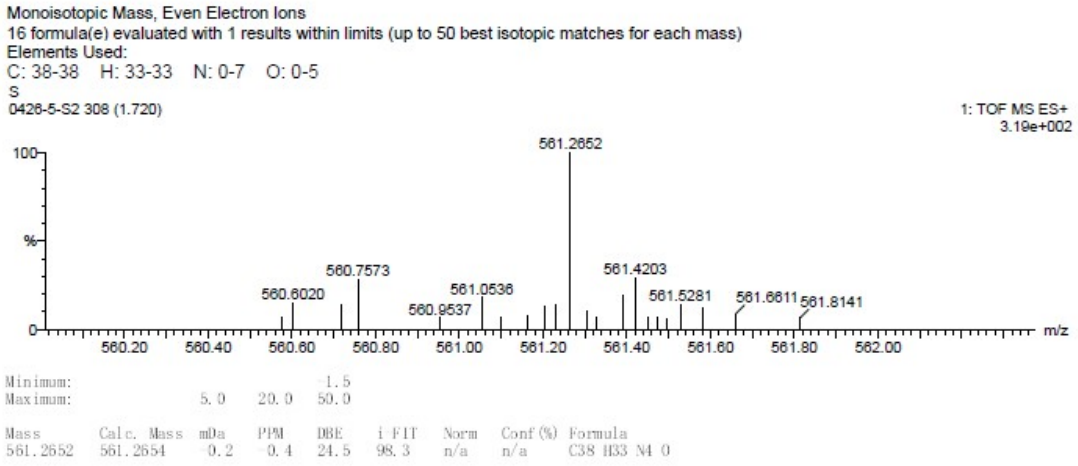

Fig. S18. HR-MS spectrum of DPABP-BI