

Supporting Information

Activatable endoplasmic reticulum-targeted NIR fluorescent probe with large Stokes shift for detecting and imaging of chymotrypsin

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1. Experimental Section

Synthesis and Characterization of ISO-OH

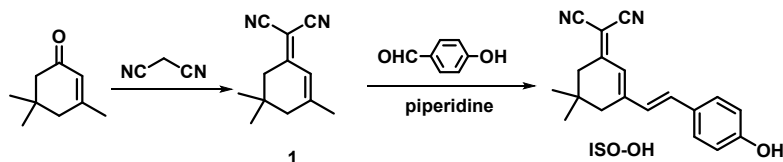


Fig. 1 Synthesis of ISO-OH

Synthesis of Compound 1. Isophorone (500 mg, 3.62 mmol), malononitrile (240 mg, 3.62 mmol) and sodium acetate trihydrate (200 mg, 136 mmol) were added to 10 mL of anhydrous ethanol. The mixture was stirred at 50 °C for 24 h. After cooling to room temperature, the solvent was removed by rotary evaporation. Then, the crude product was purified by silica gel column chromatography (PE: EA = 5: 1) to obtain Compound 1 as white crystals (322.9 mg, yield 48%). ¹H NMR (400 MHz, CDCl₃) δ 6.63 (s, 1H), 2.52 (s, 2H), 2.19 (s, 2H), 2.04 (s, 3H), 1.02 (s, 6H).

Synthesis of Compound ISO-OH. Compound 1 (400 mg, 2 mmol) and 4-hydroxybenzaldehyde (400 mg, 3 mmol) were dissolved in anhydrous ethanol (10 mL), and then piperidine (60 μL) was added. The mixture was refluxed for 4 h. After that, the solvent was removed by rotary evaporation to obtain the crude product. The crude product was purified by silica gel column chromatography (PE: EA = 3: 1) to yield ISO-OH as an orange solid (540.4 mg, 93% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 9.99 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 4.0 Hz, 2H), 6.85–6.75 (m, 3H), 2.60 (s, 2H), 2.53 (s, 2H), 1.01 (s, 6H).

2. Spectral Data of Probe ISO-Chy

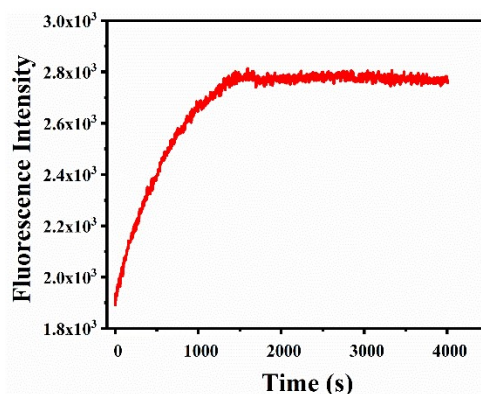


Fig. S2 Time-dependent fluorescence response of ISO-Chy (10 mM) upon addition of CHT (2 $\mu\text{g}\cdot\text{mL}^{-1}$). Conditions: DMSO/PBS (v/v=3/7, pH=7.4), λ_{ex} =540 nm, λ_{em} =675 nm, slit: 2 nm/2 nm.

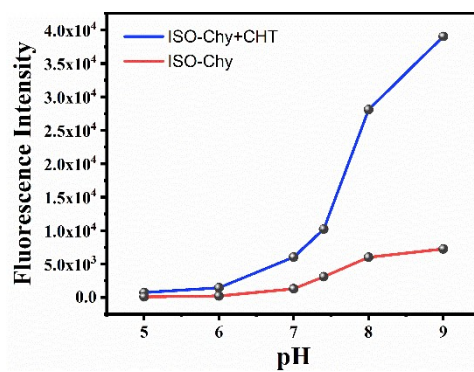


Fig. S3 Effects of pH on the fluorescence intensity of ISO-Chy (10 μM) in the presence of CHT (10 $\mu\text{g}\cdot\text{mL}^{-1}$).

3. Response Mechanism of Probe ISO-Chy to CHT

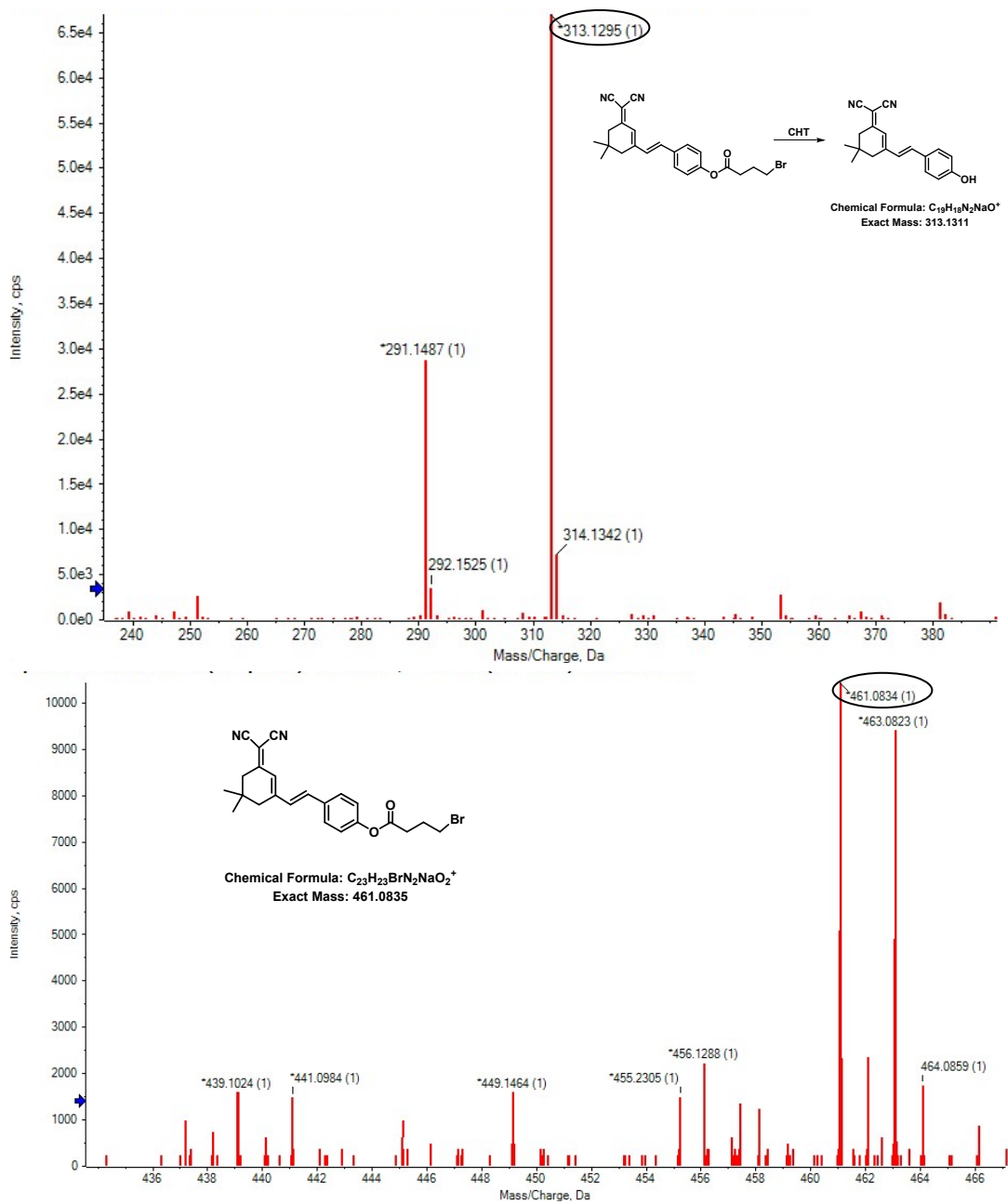


Fig. S4 HRMS spectrum of ISO-Chy with and without the addition of CHT

4. *In vitro* cytotoxicity of ISO-Chy

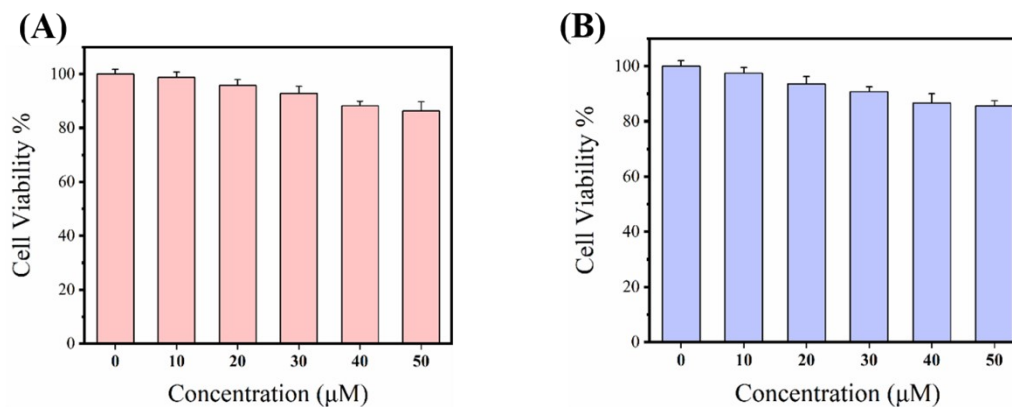


Fig. S5 Cell viability values (%) estimated by MTT proliferation test versus concentrations of ISO-Chy. (A) HepG2 cells and (B) P815 cells were cultured in the presence of 0-50 μM ISO-Chy.

5. Characterization of compounds

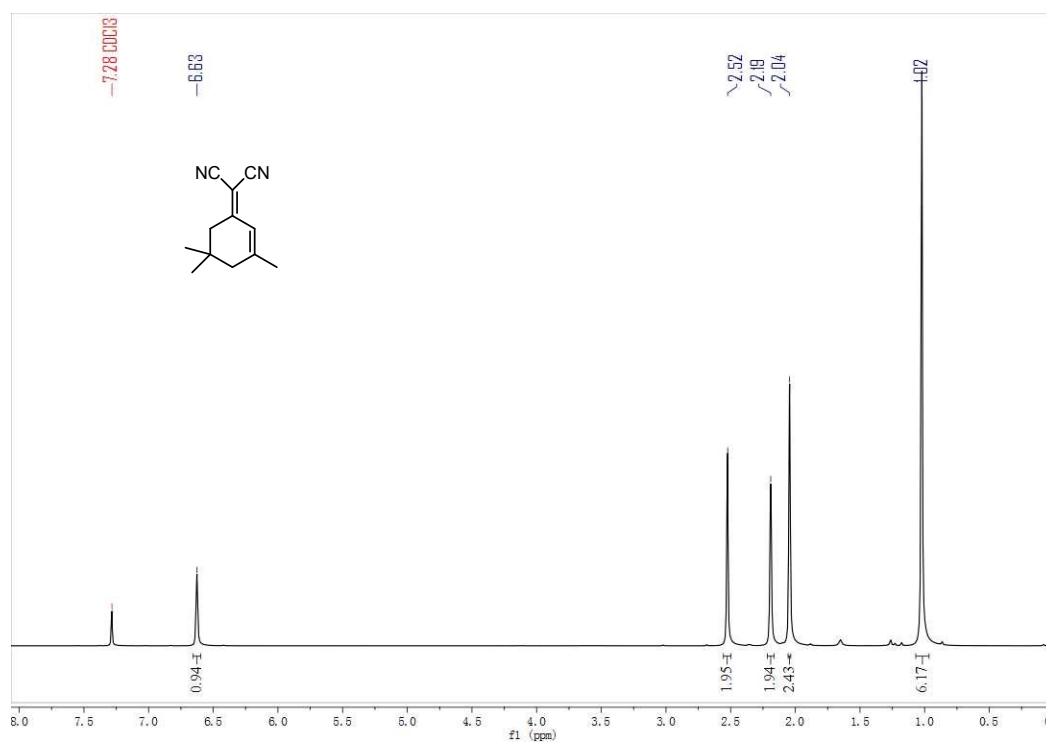


Fig. S5 ¹H NMR spectrum of compound 1 in CDCl₃

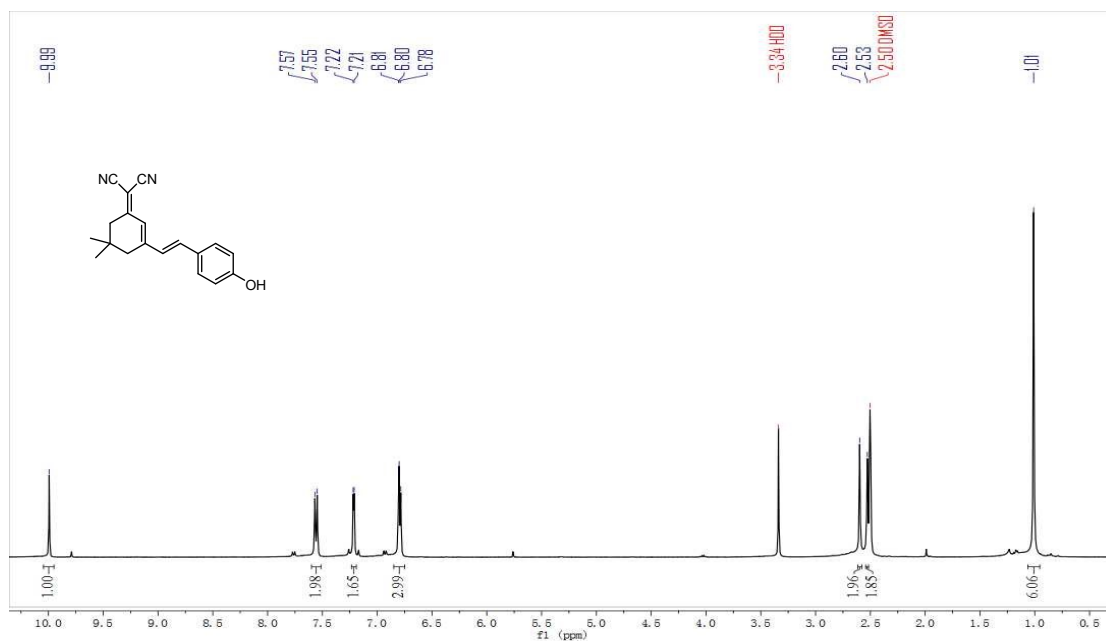


Fig. S6 ^1H NMR spectrum of compound ISO-Chy in $\text{DMSO-}d_6$

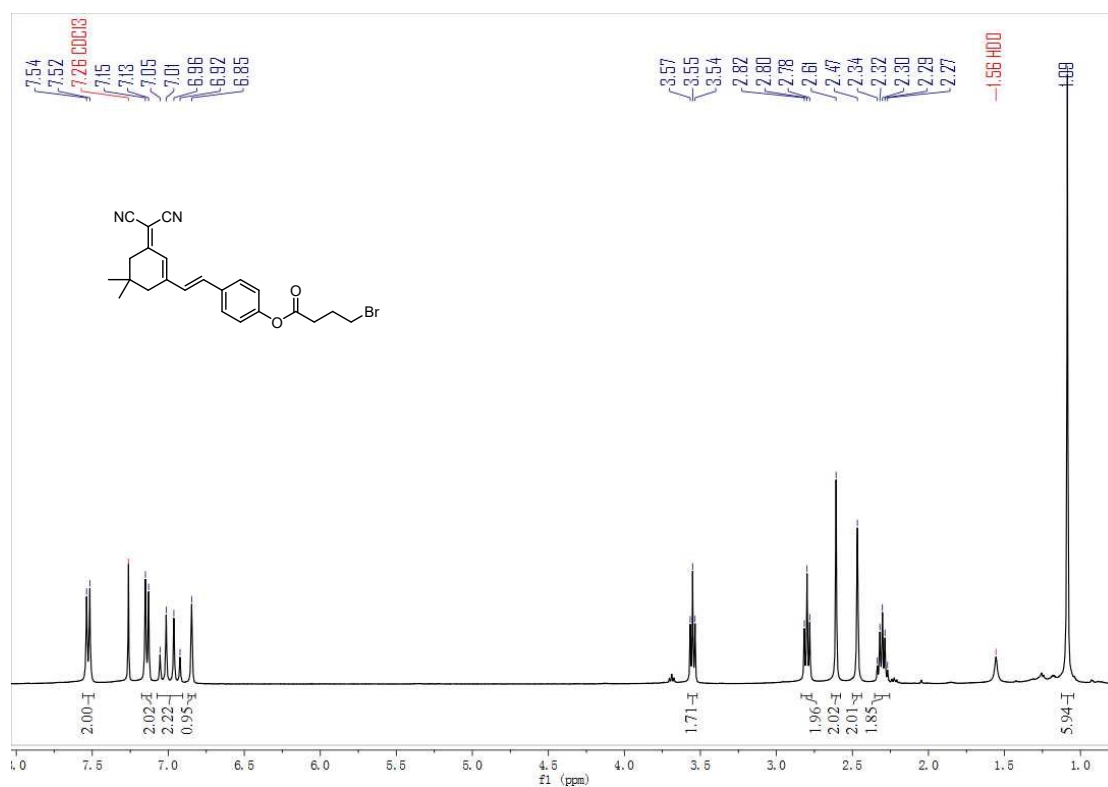


Fig. S7 ^1H NMR spectrum of ISO-Chy in CDCl_3

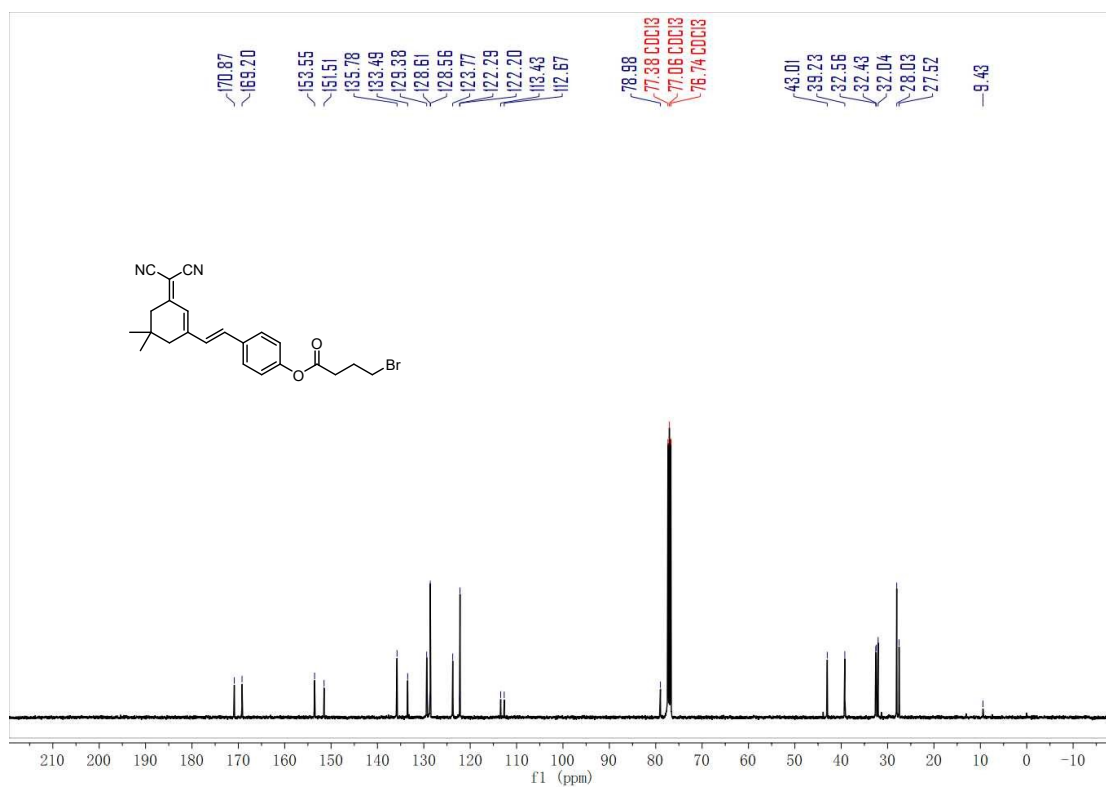


Fig. S8 ^{13}C NMR spectrum of ISO-Chy in CDCl_3

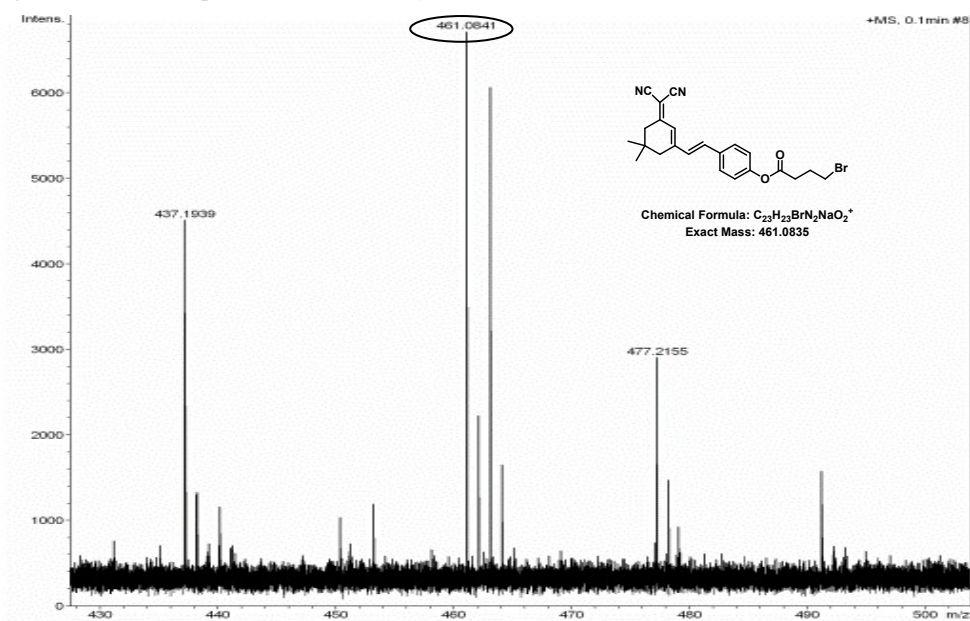


Fig. S9 HRMS spectrum of ISO-Chy