Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Selective turn-on near-infrared fluorescence probe for hypoxic tumor cell imaging

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HPLC for nitroreductase assay and cytochrome P450 metabolism assay

The purity of all tested compounds was established by HPLC to be >98.0%. HPLC analysis was performed at room temperature using a Diamonsil C_{18} (250 mm × 4.6 mm) and a mobile phase gradient from 5% CH_3CN/buffer (0.1% TFA/H_2O) to 60% CH_3CN/buffer (0.1% TFA/H_2O) for 5 min, 60% CH_3CN/buffer (0.1% TFA/H_2O) to 95% CH_3CN/buffer (0.1% TFA/H_2O) for 10min, 95% CH_3CN/buffer (0.1% TFA/H_2O) for 5min, a flow rate of 1.0 mL/min, and plotted at 440 nm. This method was used to determine the purity for the tested compounds, and also used in stability studies.

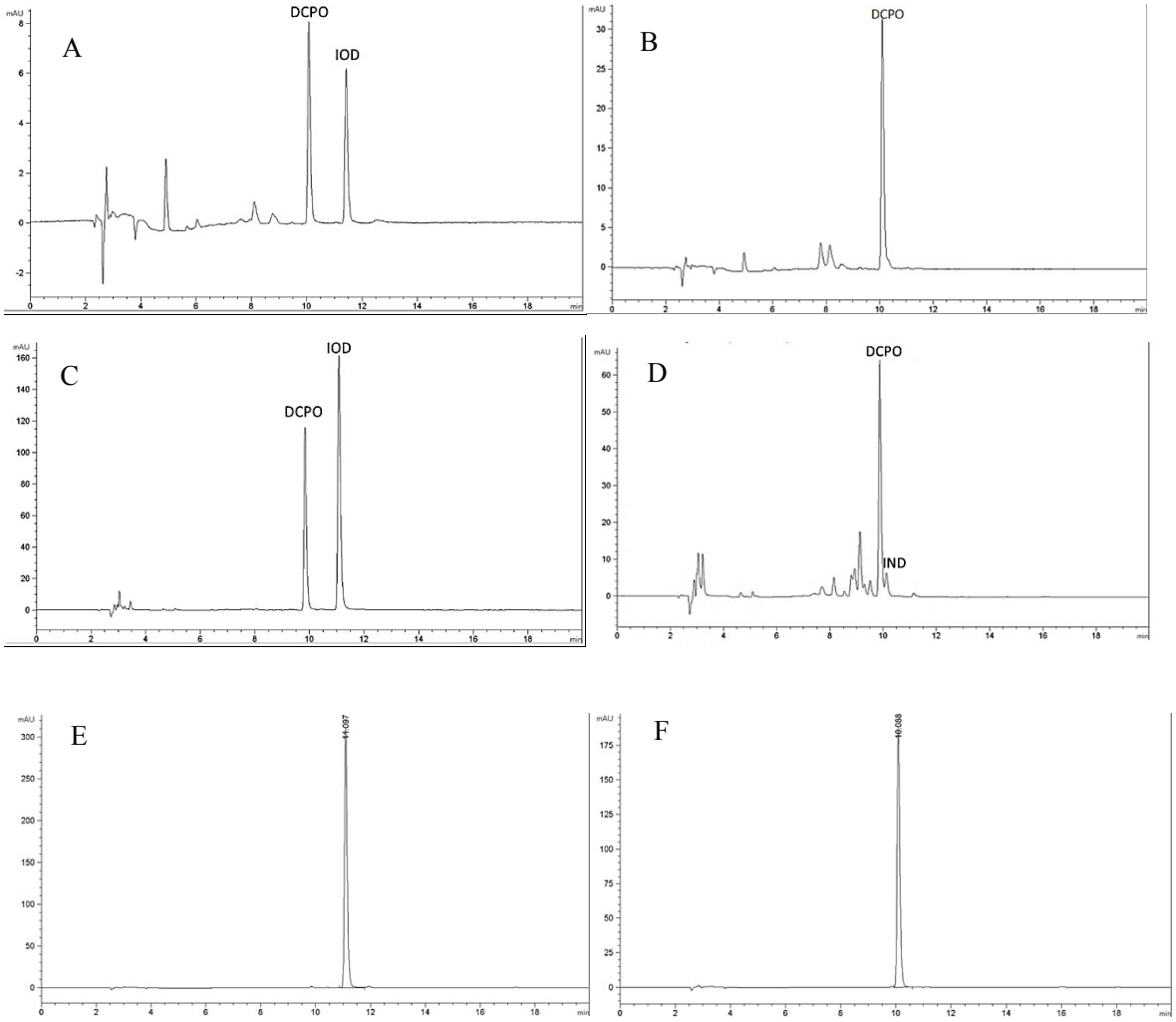


Fig. S1. HPLC for nitroreductase assay at 15 min (A,B), cytochrome P450 metabolism assay at 24 h (C,D) and purity of IOD (E) and IND (F).

In vitro cytotoxicity assay

H460, HeLa and A549 cells were seeded in 96-well plates over night and treated with test probes at concentrations of 10 μM under normoxic or hypoxic condition for 24 h. Cell viability and proliferation were assessed by MTT (n=3). The probes have no obvious toxicity at 10 μM, The cell survival rate for the tested compounds are shown in **Fig. S2**.

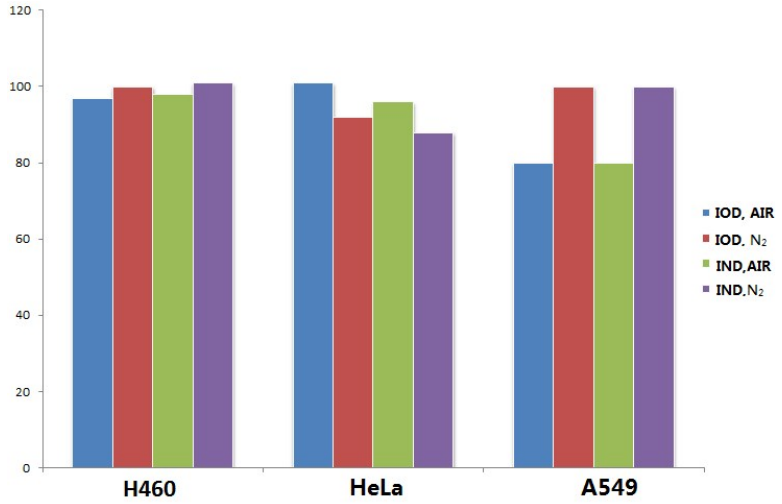
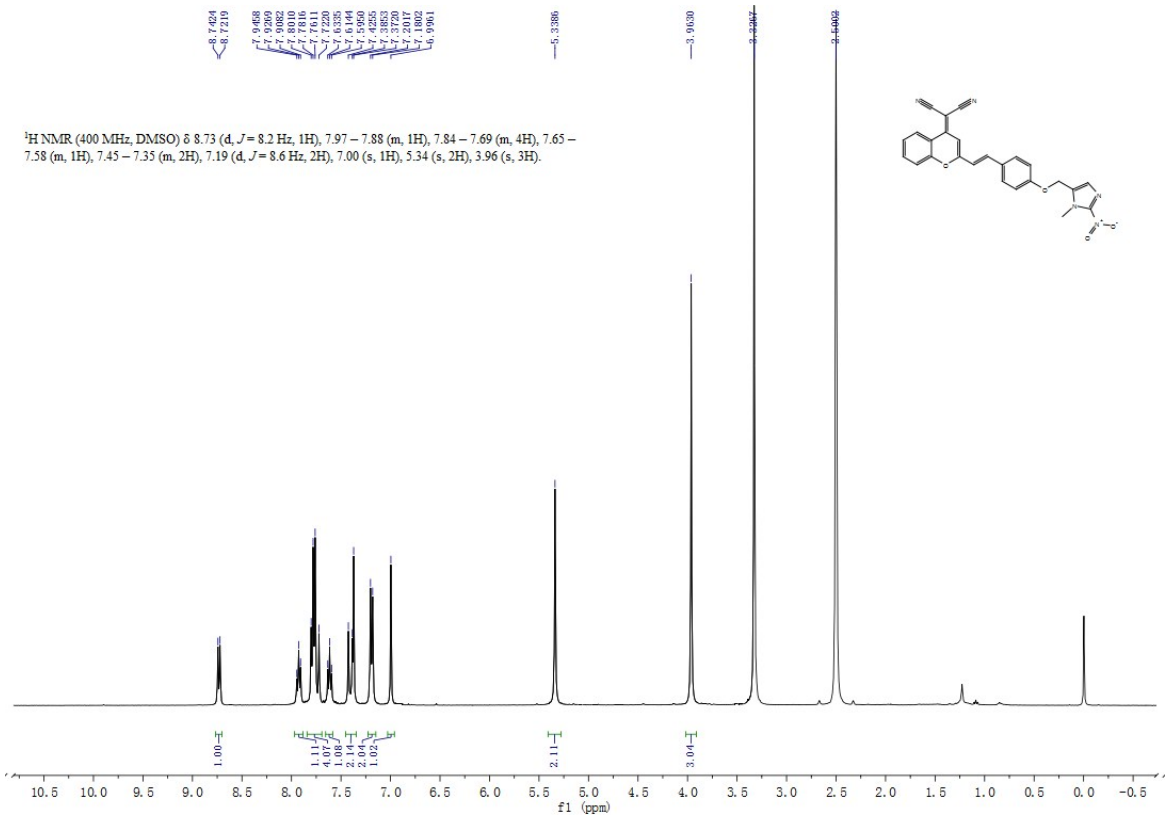
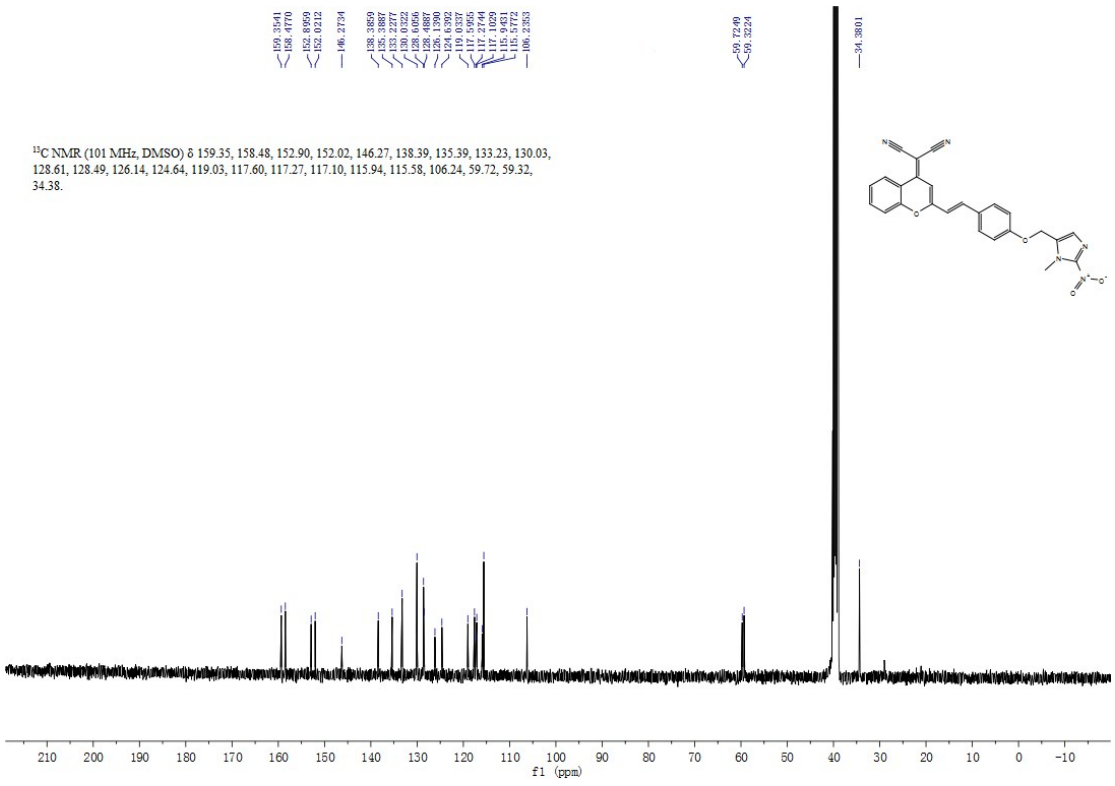


Fig. S2. In vitro cytotoxicity assay data.

^{1}H spectra and ^{13}C NMR spectra of IOD

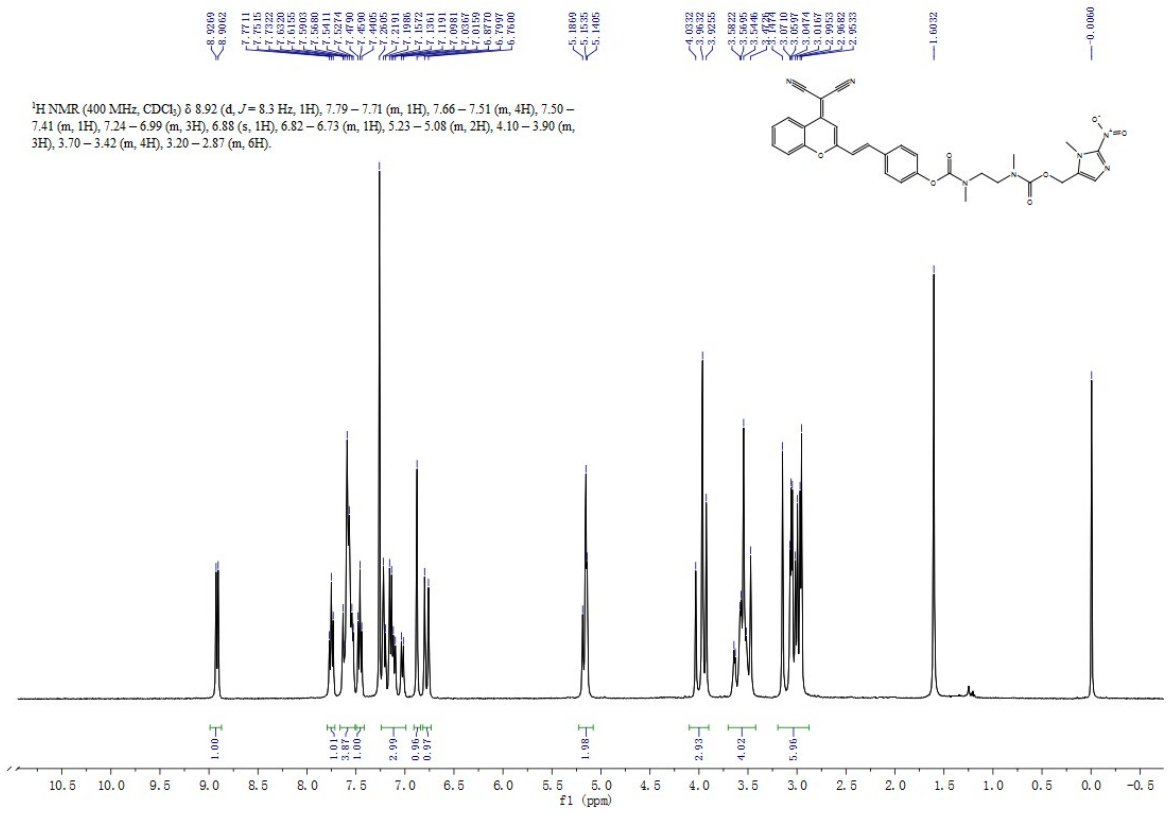


^{1}H NMR (400 MHz, DMSO) δ 8.73 (d, J = 8.2 Hz, 1H), 7.97 – 7.88 (m, 1H), 7.84 – 7.69 (m, 4H), 7.65 – 7.58 (m, 1H), 7.45 – 7.35 (m, 2H), 7.19 (d, J = 8.6 Hz, 2H), 7.00 (s, 1H), 5.34 (s, 2H), 3.96 (s, 3H).

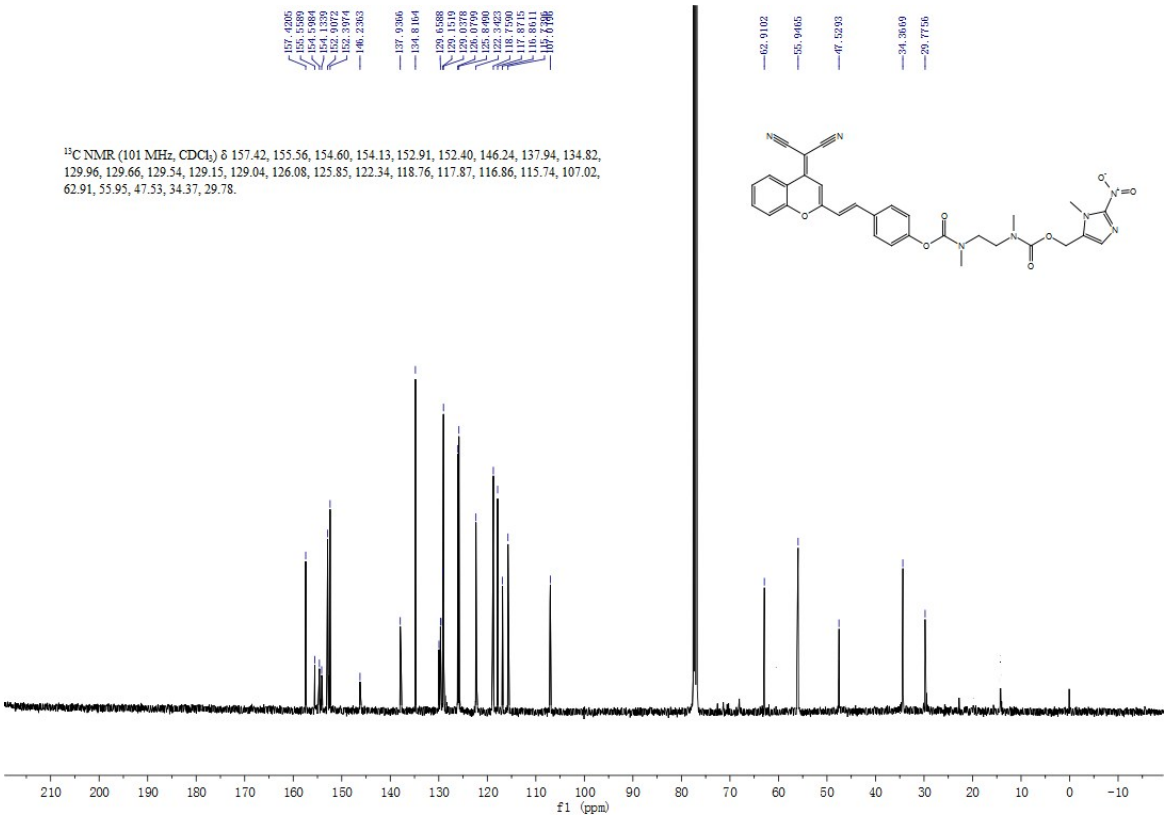


^{13}C NMR (101 MHz, DMSO) δ 159.35, 158.48, 152.90, 152.02, 146.27, 138.39, 135.39, 133.23, 130.03, 128.61, 128.49, 126.14, 124.64, 119.03, 117.60, 117.27, 117.10, 115.94, 115.58, 106.24, 59.72, 59.32, 34.38.

^{1}H spectra and ^{13}C NMR spectra of IND



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 8.3 Hz, 1H), 7.79 – 7.71 (m, 1H), 7.66 – 7.51 (m, 4H), 7.50 – 7.41 (m, 1H), 7.24 – 6.99 (m, 3H), 6.88 (s, 1H), 6.82 – 6.73 (m, 1H), 5.23 – 5.08 (m, 2H), 4.10 – 3.90 (m, 3H), 3.70 – 3.42 (m, 4H), 3.20 – 2.87 (m, 6H).



^{13}C NMR (101 MHz, $CDCl_3$) δ 157.42, 155.56, 154.60, 154.13, 152.91, 152.40, 146.24, 137.94, 134.82, 129.96, 129.66, 129.54, 129.15, 129.04, 126.08, 125.85, 122.34, 118.76, 117.87, 116.86, 115.74, 107.02, 62.91, 55.95, 47.53, 34.37, 29.78.