Supporting Information

Selective Monitoring NAD(P)H by a novel fluorescence probe to reveal reductive stress induced by natural antioxidants in live cancer cells under hypoxia

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1. Mechanism study of TCF-MQ with NADH by HRMS and ^{1}H NMR.

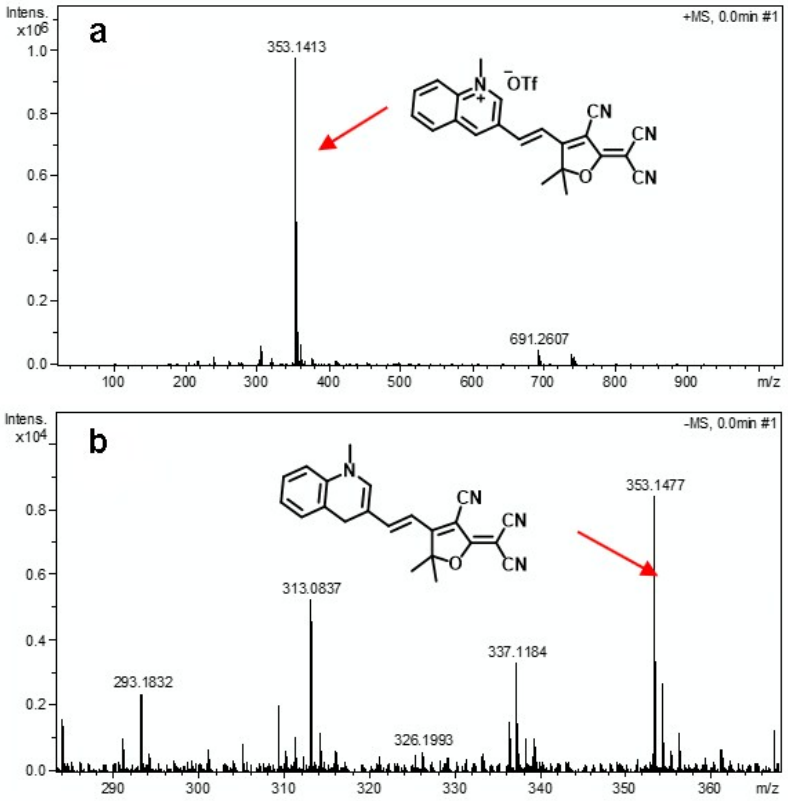

Figure S1. (a) HRMS spectra of TCF-MQ. (b) The reaction product of TCF-MQ with NADH.

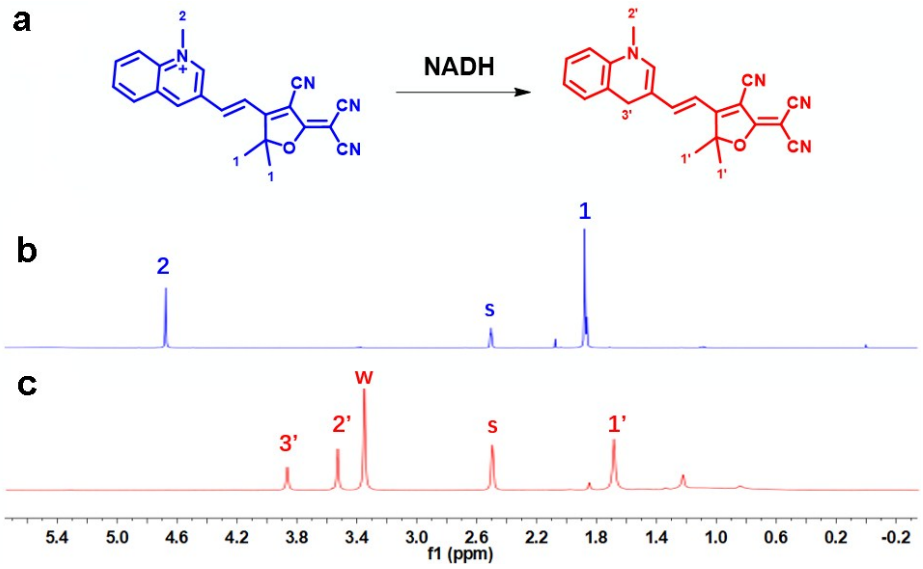

Figure S2. Partial ^{1}H NMR (400 MHz) spectra of TCF-MQ and the reaction product of TCF-MQ

with NADH in DMSO-d_6. (a) The proposed mechanism of TCF-MQ responding to NADH. (b) TCF-MQ only. (c) TCF-MQ and NADH after completion of the reaction (where S: solvent, W: water).

2. Determination of the Quantum Yield

Fluorescence quantum yield was detected by cresyl violet (Φ_F = 0.57 in methanol). The reaction product (TCF-MQH) and cresyl violet were dissolved in methanol to form a dilute solution and adjusted to give an absorbance of ~ 0.05. The emission spectra were recorded at the maximum excitation wavelength, and the integrated areas of the spectra were measured. The fluorescence quantum yield was calculated using the following equation:

$$\Phi_f = \Phi_r (A_r F_s / A_s F_r) (n_s / n_r)^2$$

Where, A is the absorbance at the maximum absorption wavelength, F is the integral area of the fluorescence spectrum, and n is the refractive index of the solvent. The subscripts r and s represent the reference and the analyte, respectively.

3. The pH influence

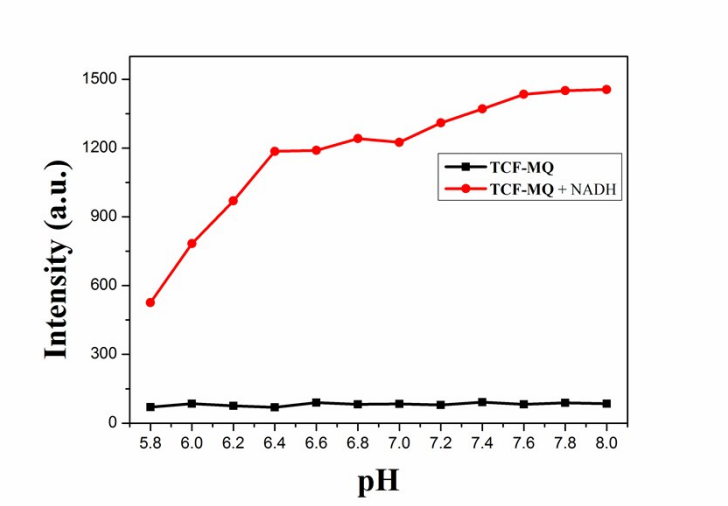

Figure S3. Fluorescence intensity changes of TCF-MQ (10 μM) at different pH values in the absence (black) or presence (red) of NADH (50 μM) in PBS buffer (10 mM, pH 7.4, 0.5% DMSO) at 37°C for 30 min. ($\lambda_{ex}/\lambda_{em}$ = 582/610 nm, Slit width: 5 nm/5 nm).

4. The fluorescence response of TCF-MQ to NADH

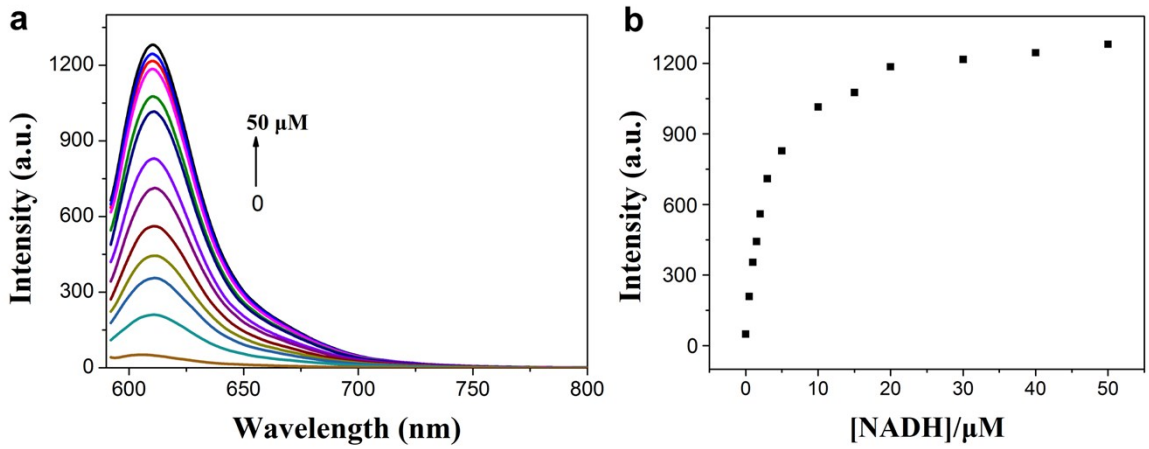

Figure S4. (a) Fluorescence response of TCF-MQ (10μM) to various concentrations of NADH (0-50 μM); (b) Fluorescence intensity at 610 nm *vs.* the NADH concentration. The measurements were performed in PBS buffer (10 mM, pH = 7.4, 0.5% DMSO) after incubation at 37°C for 30 min. ($\lambda_{ex}/\lambda_{em}$ = 582/610 nm, Slit width: 5 nm/5 nm).

5. Selectivity of TCF-MQ toward reactive oxygen species

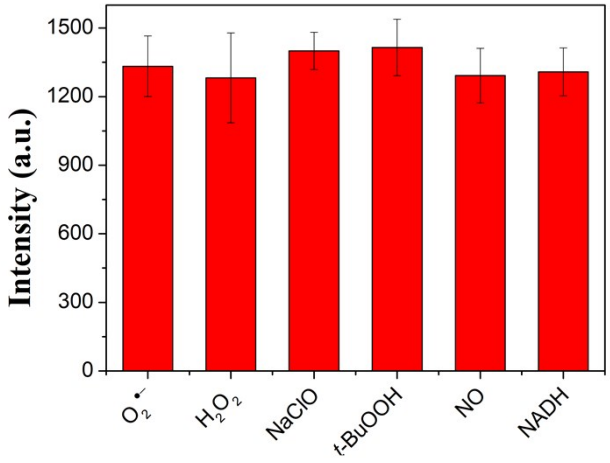

Figure S5. Fluorescence intensitiesfor the reaction product of TCF-MQ (10 μM) with NADH (50 μM) after adding reactive oxygen species. $O_2^{\bullet-}$ (500 μM), H_2O_2 (500 μM), NaClO (100 μM), *t*-BuOOH (500 μM), NO (200 μM). ($\lambda_{ex}/\lambda_{em}$ = 582/610 nm, Slit width: 5 nm/5 nm).

6. Cytotoxicity Assay

HepG2 cells (1.5×10^4 cells/well) were added to 96-well plates with 200 μL DMEM culture medium and incubated for 24 h at 37 °C. Then the cells supernatant was removed and incubated with different concentrations of TCF-MQ for 18 h. 20 μL MTT solution (5 mg/mL) was added into each well. After 4 h, the supernatant was discarded, and 100 μL dimethyl sulphoxide (DMSO) was added to each well to dissolve the formazan crystals. Absorbance was recorded at a wavelength of 490 nm with a microtiter plate reader.

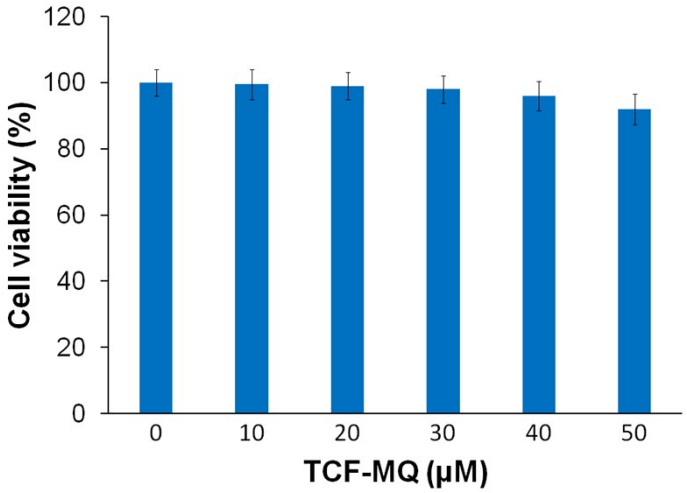

Figure S6. Cell viability estimated by MTT assay. HepG2 cells were incubated with 0-50 μM TCF-MQ for 18 h, then the cell viabilities were determined by an MTT assay.

7. Photo-bleaching test of the reaction product of TCF-MQ with NAD(P)H

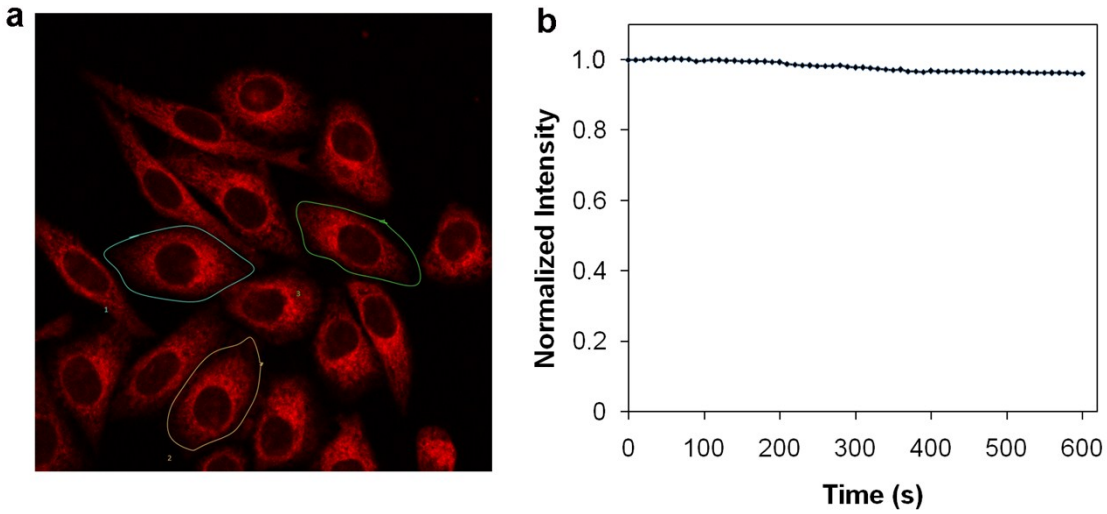

Figure S7. Test of photostability of TCF-MQ (10 μM) (a) HepG2 cells were incubated with TCF-MQ (10 μM) for 20 min, the fluorescence images (0-600 s) were achieved by means of time-sequential scanning. (b) Normalized fluorescence intensity of the three selected regions of (a) from 0 to 600s.

8. Fluorescence analysis of NAD(P)H

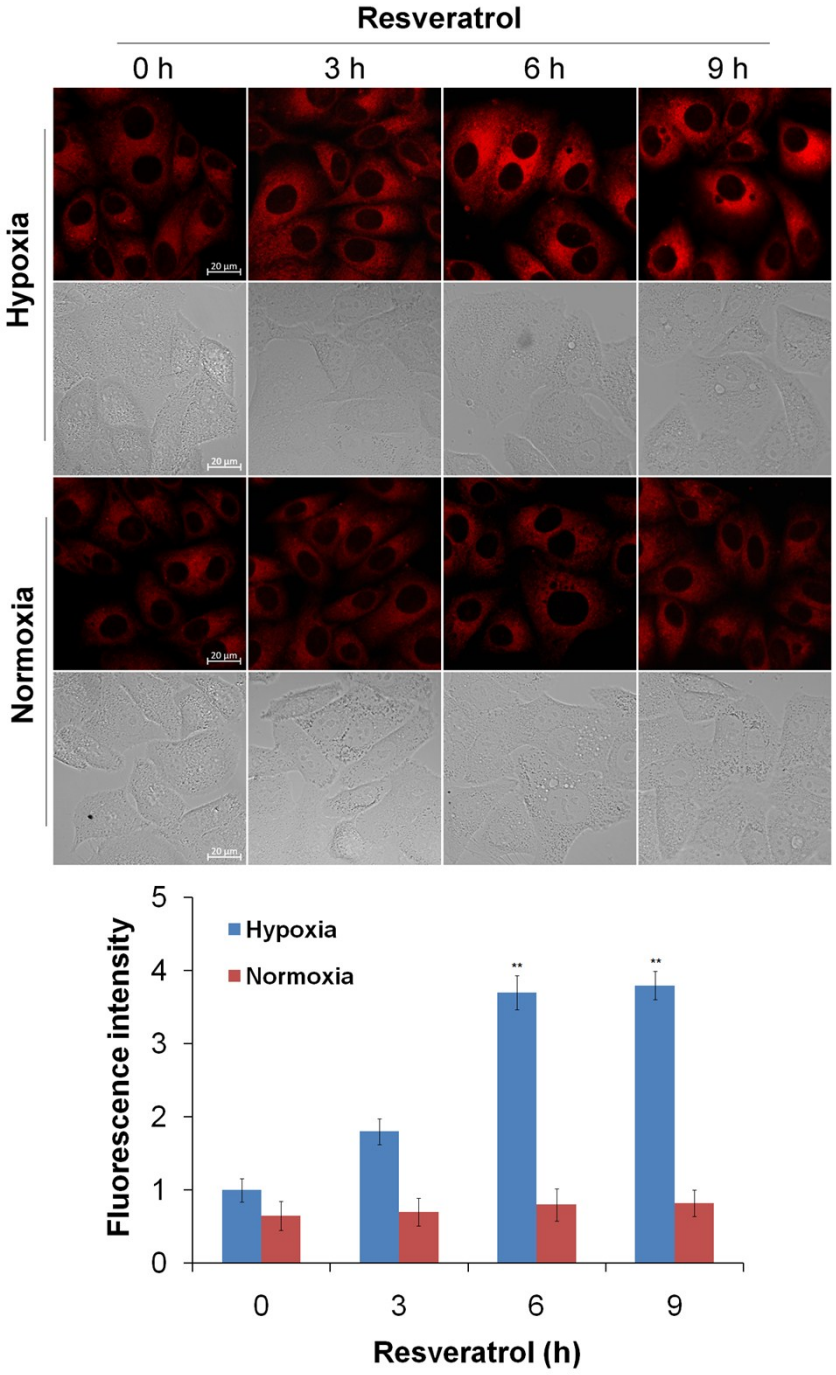

Figure S8. Detection of NAD(P)H levels induced by resveratrol. HepG2 cells were exposed to 10 μg/mL resveratrol for 0-9 h under hypoxic conditions (1%O_2) and normoxic conditions (20%O_2), then the cells were incubated with 10 μM of the TCF-MQ probe for 20 min before the fluorescence images were obtained using confocal microscopy. (**p<0.01, t test). The scale bar in all fluorescence images of cells is 20 μm.

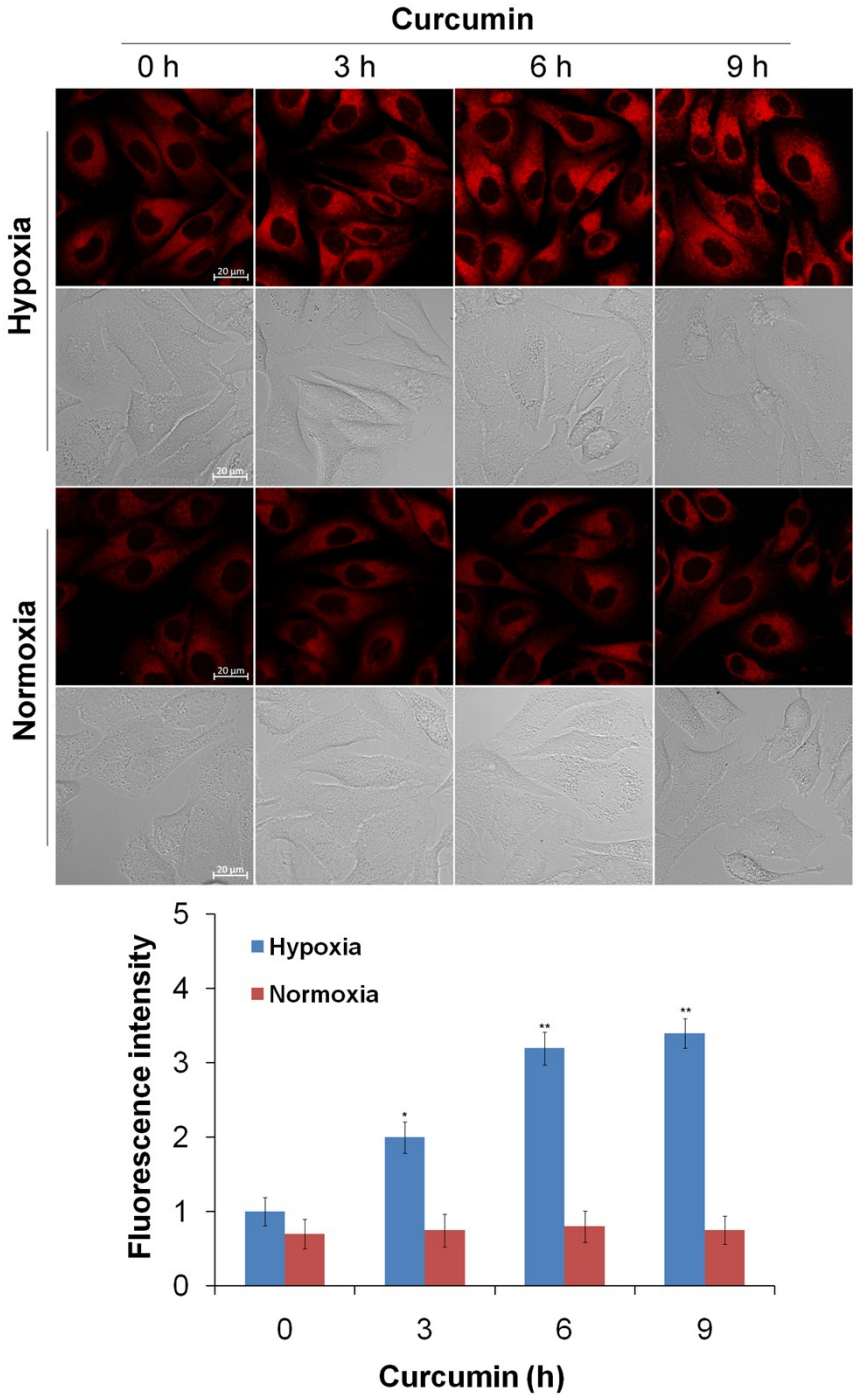

Figure S9. Detection of NAD(P)H levels induced by curcumin. HepG2 cells were exposed to 10 μg/mL curcumin for 0-9 h under hypoxic conditions (1%O_2) and normoxic conditions (20%O_2), then the cells were incubated with 10 μM of the TCF-MQ probe for 20 min before the fluorescence images were obtained using confocal microscopy. (*p<0.05, **p<0.01, t test). The scale bar in all fluorescence images of cells is 20 μm.

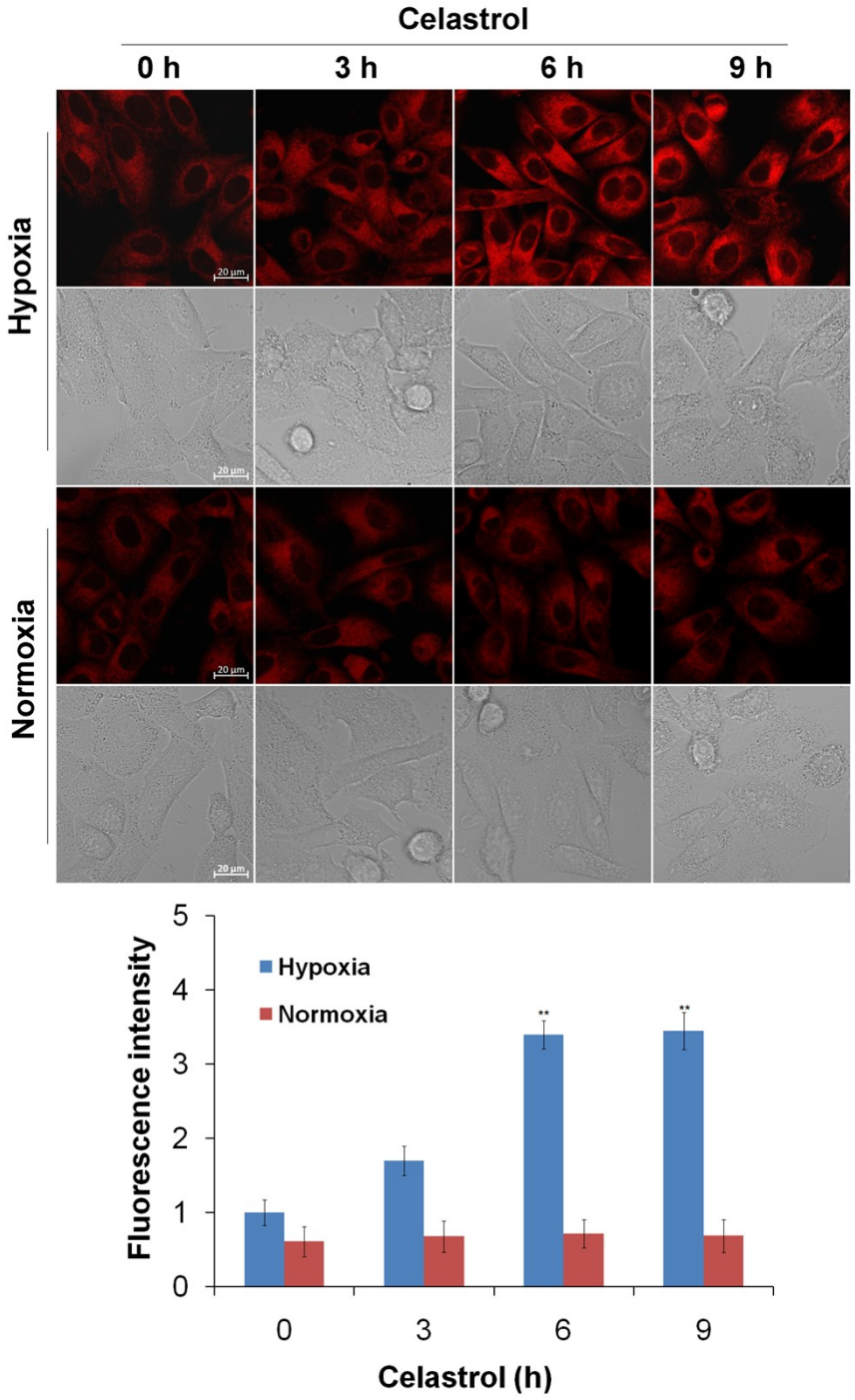

Figure S10. Detection of NAD(P)H levels induced by celastrol. HepG2 cells were exposed to 2 μg/mL celastrol for 0-9 h under hypoxic conditions (1%O_2) and normoxic conditions (20%O_2), then the cells were incubated with 10 μM of the TCF-MQ probe for 20 min before the fluorescence images were obtained using confocal microscopy. (**p<0.01, t test). The scale bar in all fluorescence images of cells is 20 μm.

9. Fluorescence analysis of H_2O_2

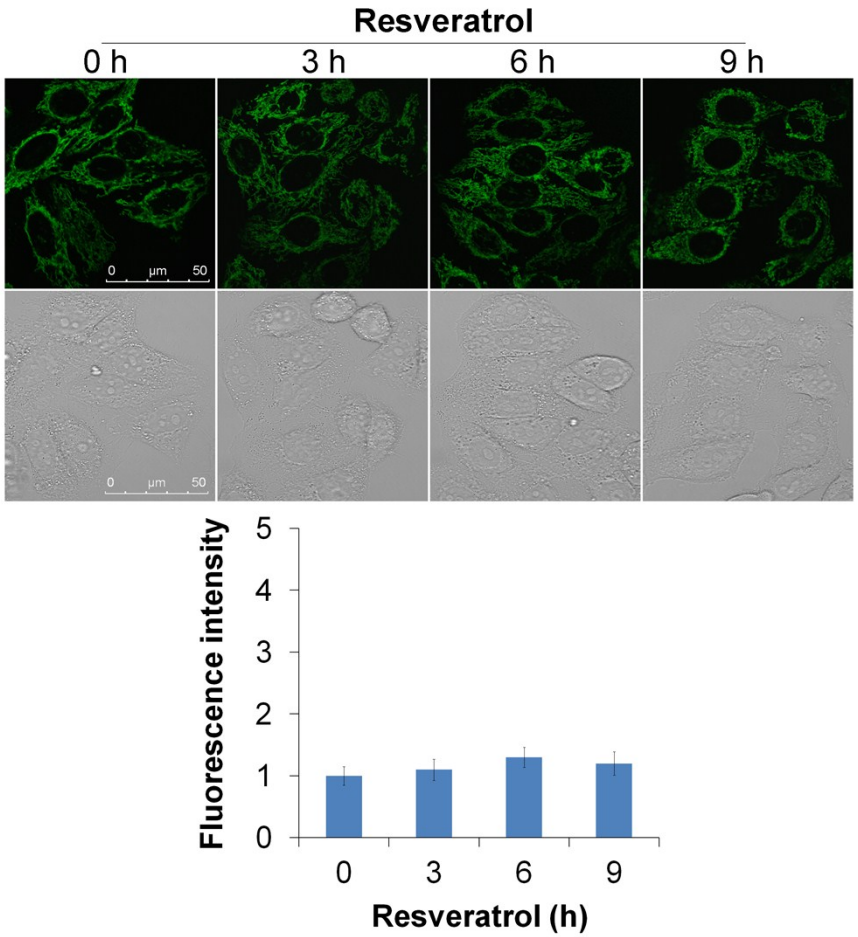

Figure S11. Analysis of H_2O_2 levels induced by resveratrol. HepG2 cells were exposed to 10 μg/mL resveratrol for 0-9 h under hypoxic conditions (1%O_2), and then, the cells were incubated with 10 μM of the H_2O_2 probe for 15 min before the fluorescence images were obtained using confocal microscopy. The scale bar in all fluorescence images of cells is 50 μm.

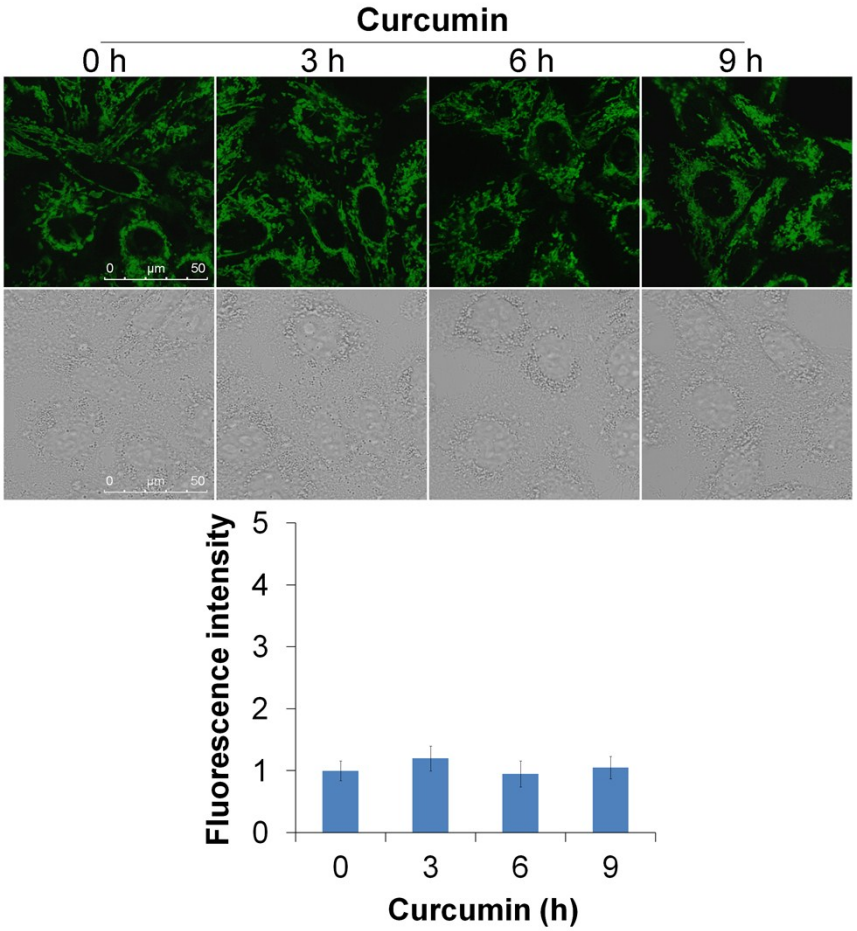

Figure S12. Analysis of H_2O_2 levels induced by curcumin. HepG2 cells were exposed to 10 μg/mL curcumin for 0-9 h under hypoxic conditions (1%O_2), and then, the cells were incubated with 10 μM of the H_2O_2 probe for 15 min before the fluorescence images were obtained using confocal microscopy. The scale bar in all fluorescence images of cells is 50 μm.

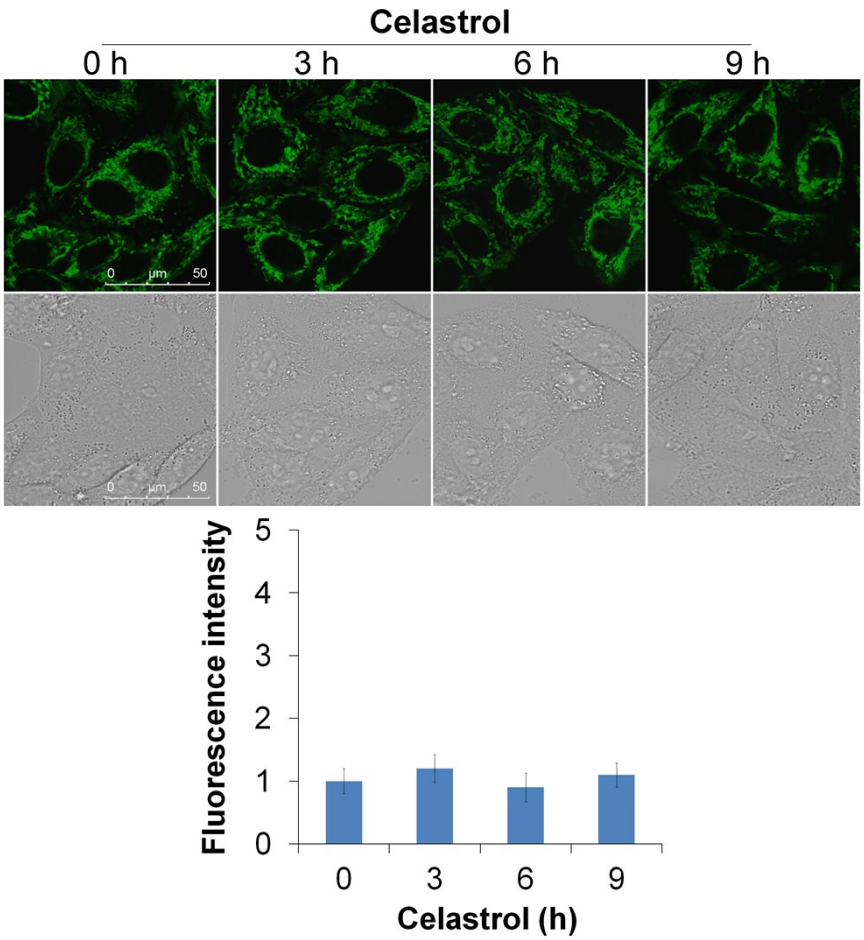

Figure S13. Analysis of H_2O_2 levels induced by celastrol. HepG2 cells were exposed to 10 μg/mL celastrol for 0-9 h under hypoxic conditions (1%O_2), and then, the cells were incubated with 10 μM of the H_2O_2 probe for 15 min before the fluorescence images were obtained using confocal microscopy. The scale bar in all fluorescence images of cells is 50 μm.

10. Spectra of compound 1, compound 2 and TCF-MQ.

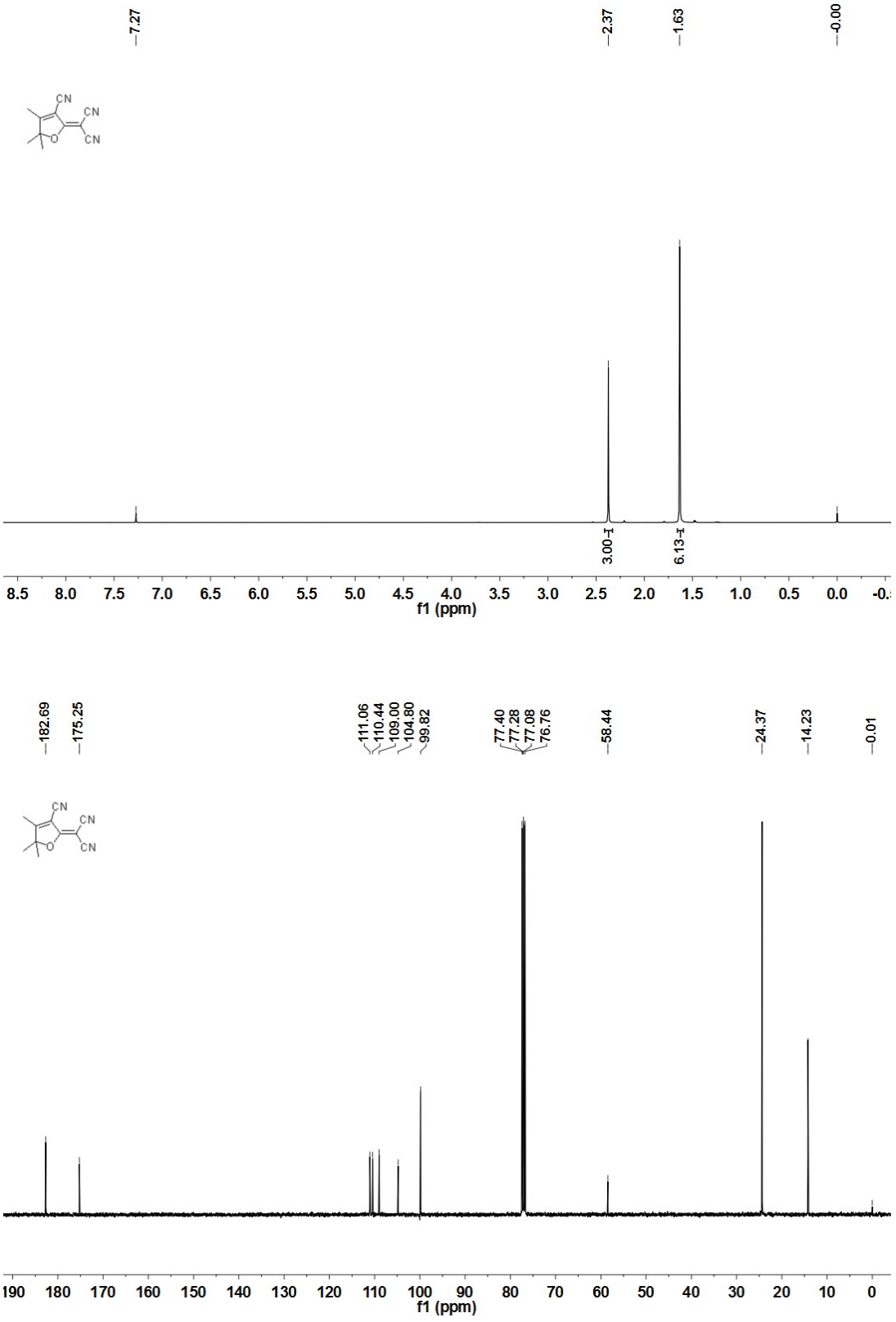

Figure S14. ^{1}H and ^{13}C NMR spectra of compound **1** in $CDCl_3$.

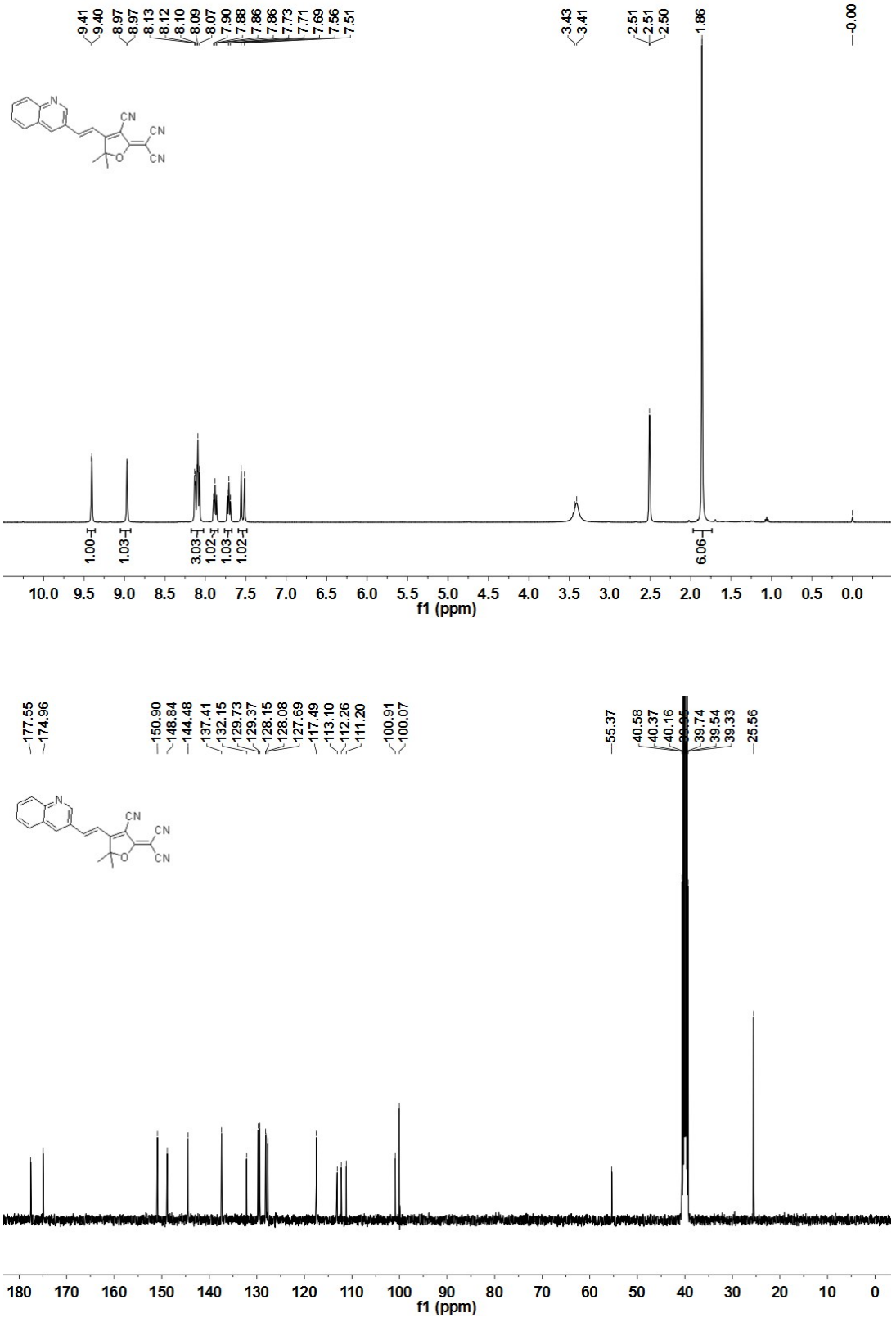

Figure S15. ^{1}H and ^{13}C NMR spectra of compound **2** in DMSO-d_6.

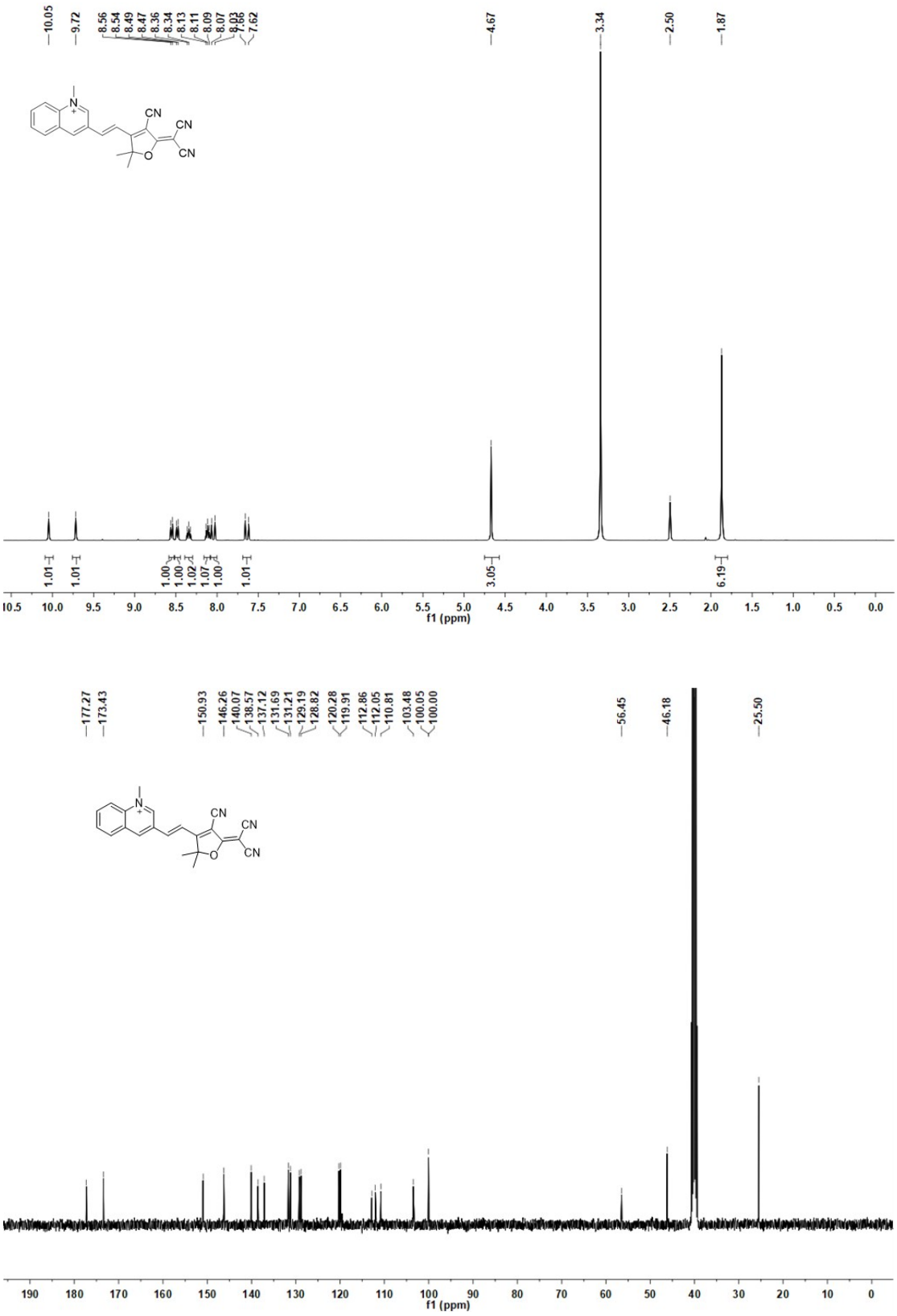

Figure S16. ^{1}H and ^{13}C NMR spectra of **TCF-MQ** in DMSO-d_6.