Supplementary Information

A distinctive mitochondria-targeting and *in situ* activated near-infrared fluorescent probe for visualizing sulfur dioxide derivatives and their fluctuation *in vivo*

Lintao Zeng,[a,b,*] Tianhong Chen,[a,b] Bao-Quan Chen,[b] Hou-Qun Yuan,[c] Ruilong Sheng,[d,*] Guang-Ming Bao [c,*]

[a] *College of Chemistry and Materials Science, Hubei Engineering University, Hubei Xiaogan 432100, P. R. China. E-mail: zlt1981@126.com (L. Zeng).*

[b] *Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, Tianjin University of Technology, Tianjin, 300384, PR China.*

[c] *School of Animal Science and Technology, Jiangxi Agricultural University, Nanchang 330045, PR China. E-mail: bycb2005@gmail.com (G.-M. Bao).*

[d] *CQM-Centro de Quimica da Madeira, Universidade da Madeira, Campus da Penteada, 9000-390, Funchal, Madeira, Portugal. Fax: (+351) 291705254; E-mail: ruilong.sheng@staff.uma.pt (R. Sheng).*

Contents

1. Experimental details……S2

2. Determination of the detection limit……S3

3. Investigation of sensing mechanism……S3

4. Cytotoxicity assay……S4

5. The photostability of **DCQN** for $HSO_3^-$and fluorescence imaing in living cells…S5

6. Fluorescence imaging of zebrafish……S6

7. Structure characterization……S7

1. Experimental details

1.1 Spectroscopic measurements.

For UV-vis and fluorescence titrations and optical responses of **DCQN** toward various analytes, stock solution (1 mM) of **DCQN** were prepared in HPLC grade DMSO. Stock solutions of HSO_3^- and other analytes (10 mM) were prepared in distilled water. For optical measurements, **DCQN** was diluted to 10 μM in DMSO/HEPES buffer solution (v/v = 1/9, 10 mM, pH 7.4), and 2.0 mL of the resulting solution was placed in a quartz cell of 1.0 cm optical path length each time. The UV-vis and fluorescence titrations were recorded using spectrophotometers upon addition of analytes at room temperature.

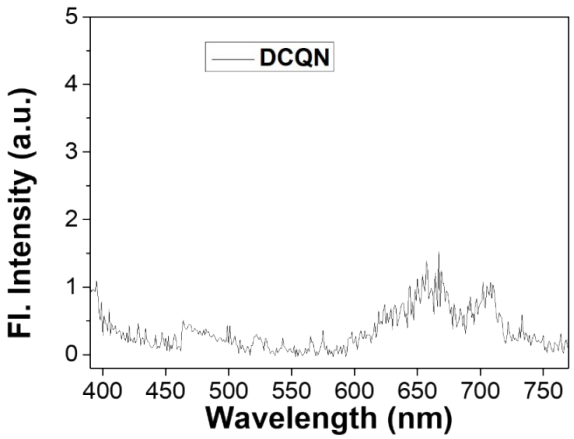

Fig. S1. The fluorescence spectra of **DCQN** (10 μM) in DMSO/HEPES buffer solution (v/v = 1/9, pH 7.4). The excitation wavelength was 380 nm. Slits: 5/5 nm.

1.2 Determination of the quantum yield.

Fluorescence quantum yield was measured by using Cy 5.5 (Φ = 0.28 in PBS buffer)[1] as a fluorescence reference. The Cy 5.5 and reaction product (DCQN-HSO_3) were dissolved in PBS and adjusted to give an absorbance of ca. 0.05. Then, the fluorescence emission spectrum was recorded at the maximum excitation wavelength, and the integrated areas of the spectra were calculated. The fluorescence quantum yield was determined according to the following equation:

$$\Phi_{FS} = \Phi_{FR} (A_R F_S / A_S F_R) (\eta_S / \eta_R)^2$$

Where Φ is the fluorescence quantum yield, A is the absorbance at the maximum absorption wavelength, F is the integral area of the fluorescence spectrum, and η is the refractive index of the solvent. The subscripts S and R represent the analyteand the reference.

2. Determination of the detection limit.

The calibration curve was first obtained from the plot of fluorescence F_{660} as a function of HSO_3^- level. The regression curve equation was obtained for the lower concentration part.

$$\text{Detection limit (LOD)} = 3 \times \sigma/k$$

where k is the slope of the curve equation, and σ represents the standard deviation for the fluorescence intensity of the probe in the absence of phosgene. $F_{660} = 3.7323 + 23.9522 \times [HSO_3^-]$ ($R^2 = 0.9976$)

$$\text{Detection limit (LOD)} = 3 \times 0.0000001916/23.9522 = 24 \text{ nM}$$

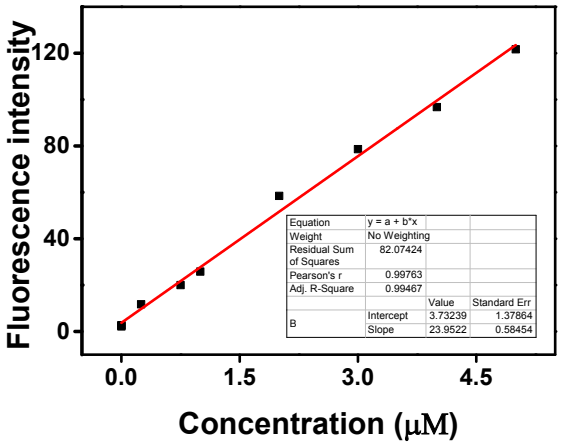

Fig. S2. The calibration curve of fluorescence intensity (F_{660}) of **DCQN** (1 μM) as a function of HSO_3^- concentration.

3. Investigation of sensing mechanism.

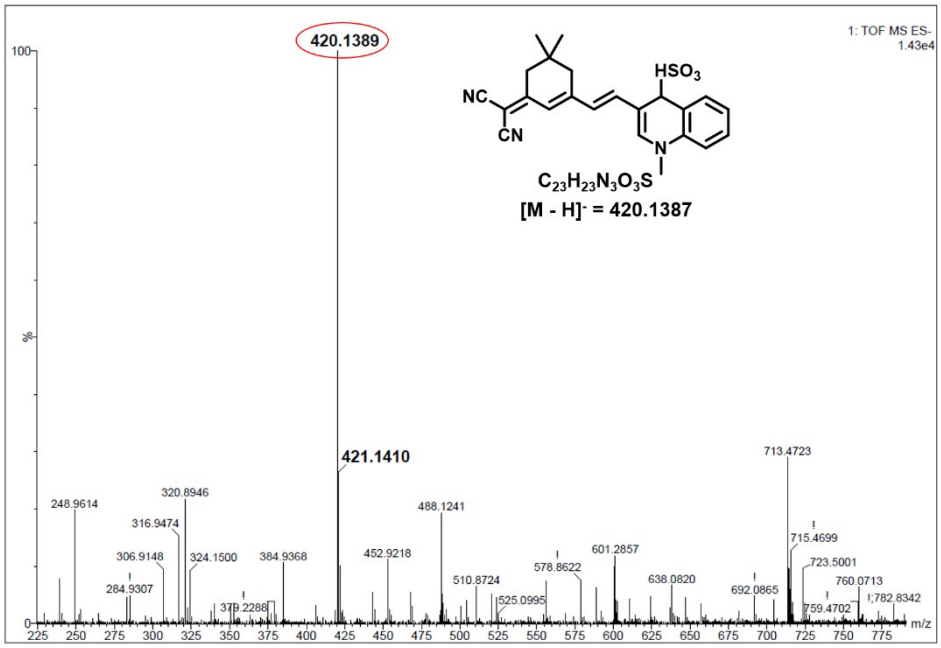

Fig. S3. HR-MS spectrum of **DCQN-HSO$_3$**.

4. Cytotoxicity assay.

The cytotoxicity of **DCQN** in human breast cancer (MCF-7) cells was evaluated by using the Cell Counting Kit-8 (Shanghai Biyuntian Bio-Technology Co., Ltd.). MCF-7 cells were grown in 96-well plates (Corning) at 5000 cells per well. After the cells completely attached to the plates for 24 h, each well was washed with 100 μL PBS, and then incubated with various concentrations of **DCQN** (2.5, 5, 10, 15, 20, and 25 μM) for 24 h. Afterwards, each well was washed with 100 μL PBS and added 100 μL serum-free DMEM containing 10% CCK-8, and further incubated for 1 h, Finally, the absorbance at 450nm was determined by a plate reader (BioTek: Gene Co., Ltd).

5. The photostability of DCQN for HSO_3^- and fluorescence imaing in living cells.

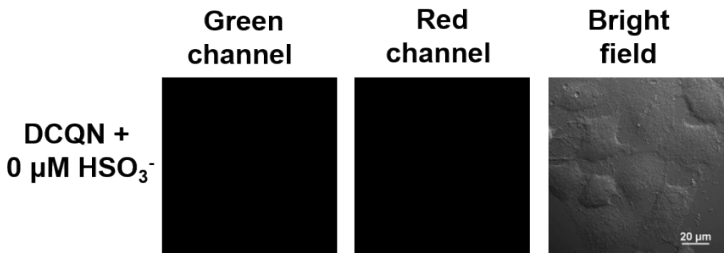

Fig. S4. The images of MCF-7 cells after incubation with DCQN (10 μM) for 30 min in the absence of bisulfate.

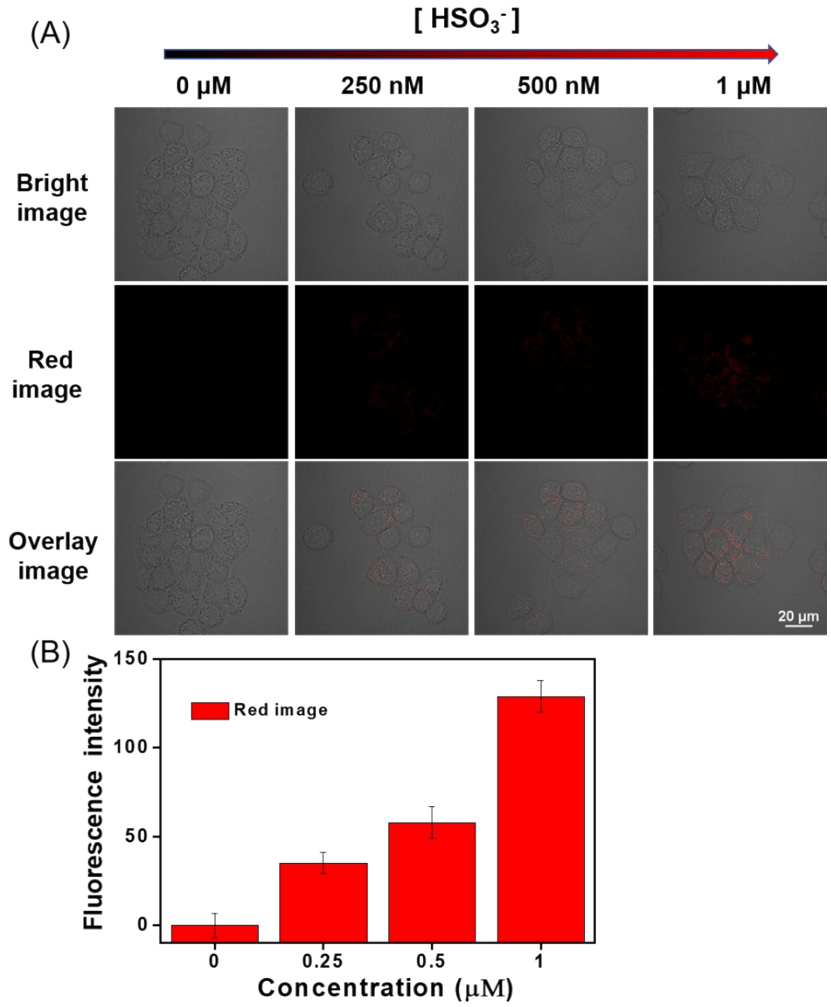

Fig. S5. Confocal images of HeLa cells incubated with DCQN (10 μM) at 37 °C for 30 min, and then treated with different concentrations of HSO_3^- (0 − 1 μM) for 10 min. Cell images were acquired with λex/λem of 543/648−703 nm. Scale bar: 20 μm. (B) Statistical analysis based on peak fluorescence intensity of HeLa cells. Error bars are ± SD, n = 5.

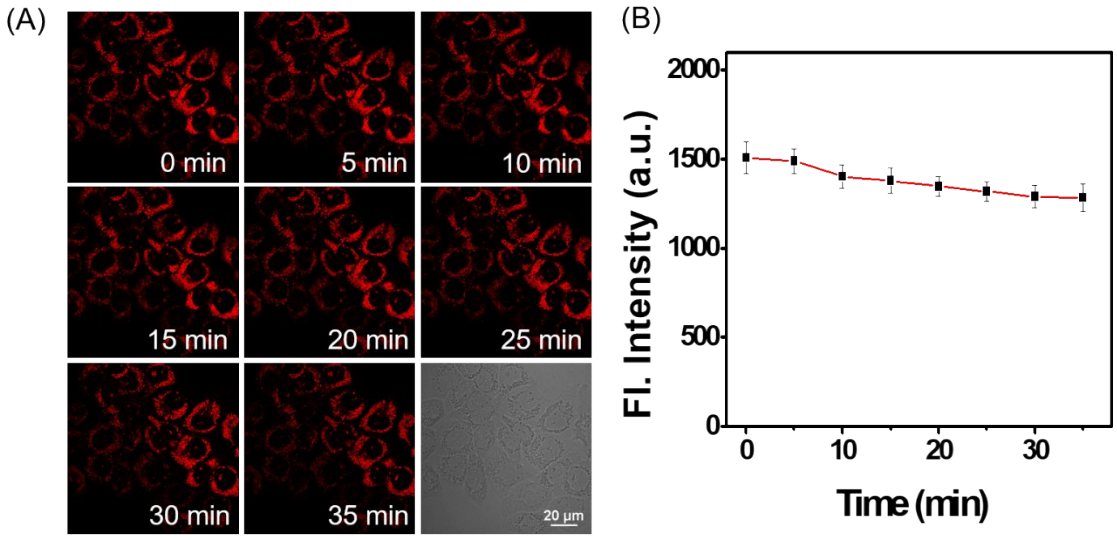

Fig. S6. (A) Photostability of **DCQN** for HSO_3^- in living HeLa cells. HeLa cells were incubated with **DCQN** (10 μM) for 30 min at 37 °C, and then incubated with HSO_3^- (60 μM) for another 10 min, and moved on a confocal microscope for irradiating by continuous scanning. (B). The mean fluorescence intensity at different time. Irradiation time: 4.6 s per scan. $\lambda_{ex}/\lambda_{em}$ of 543/648−703 nm. Scale bar 20 μm. Error bars are ± SD, n = 5.

6. Fluorescence imaging of zebrafish.

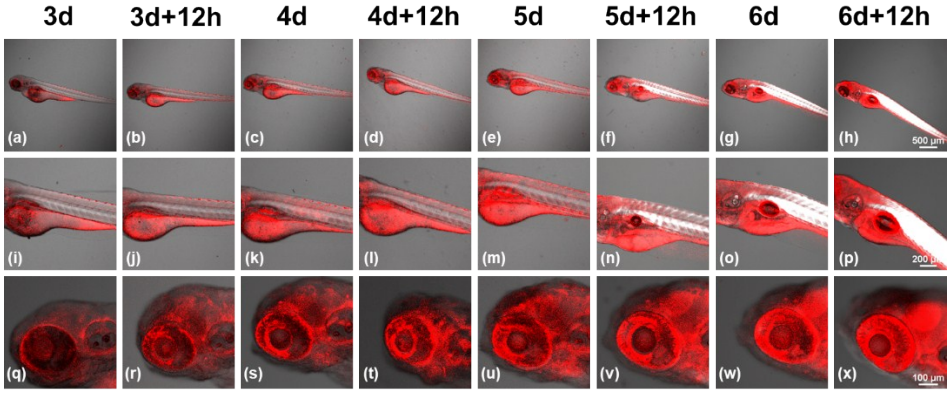

Fig. S7. (a-h) merged of red/bright image from figure 6, (i-x) enlarge of merged image.

7. Structure characterization.

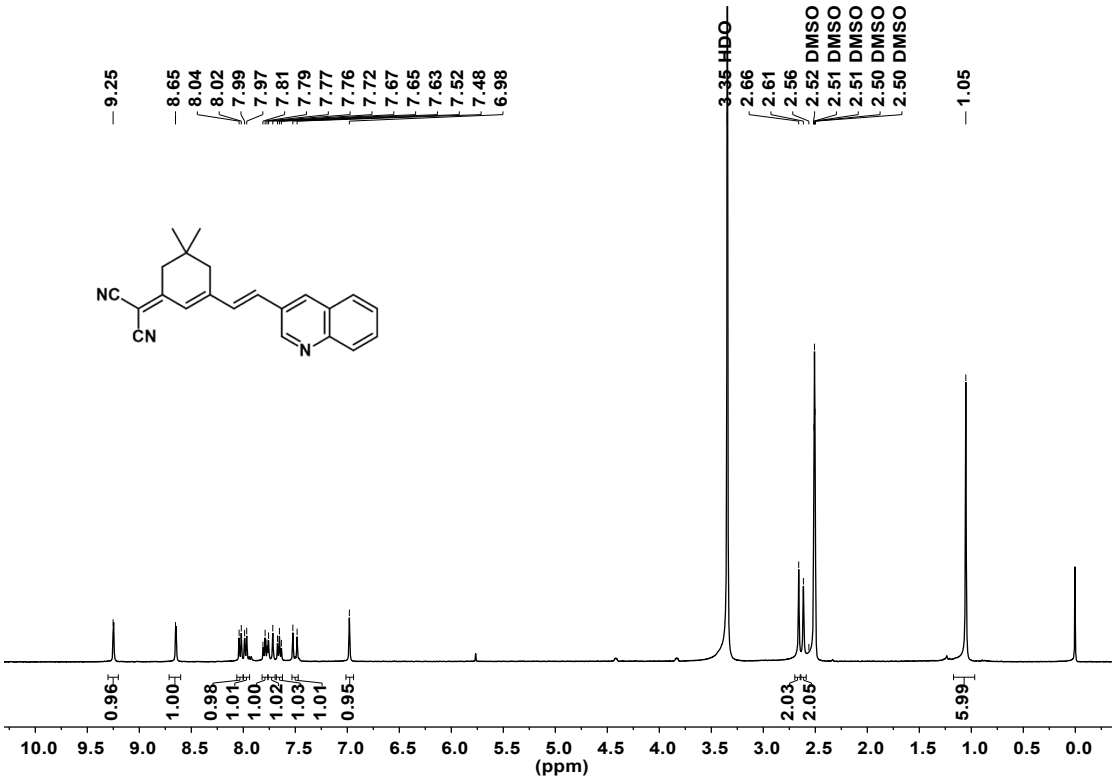

Fig. S8. ^{1}H NMR spectrum of **DCIQ** in DMSO-d_6 (400 MHz).

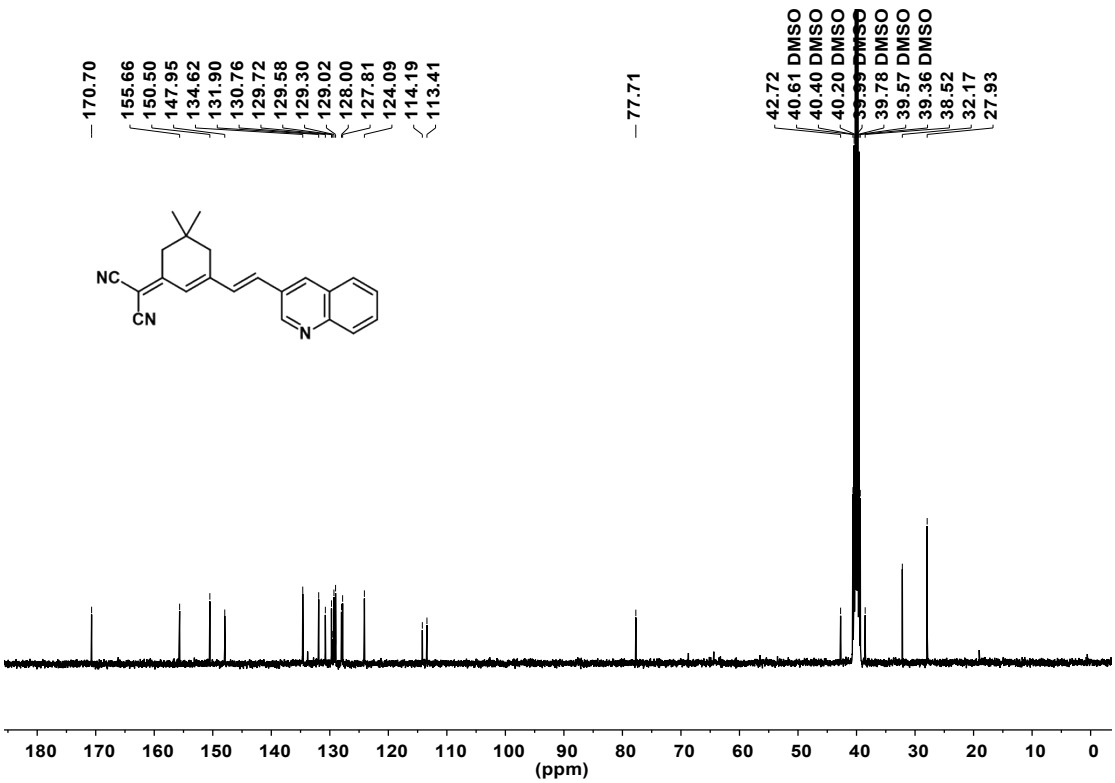

Fig. S9. ^{13}C NMR spectrum of **DCIQ** in DMSO-d_6 (100 MHz).

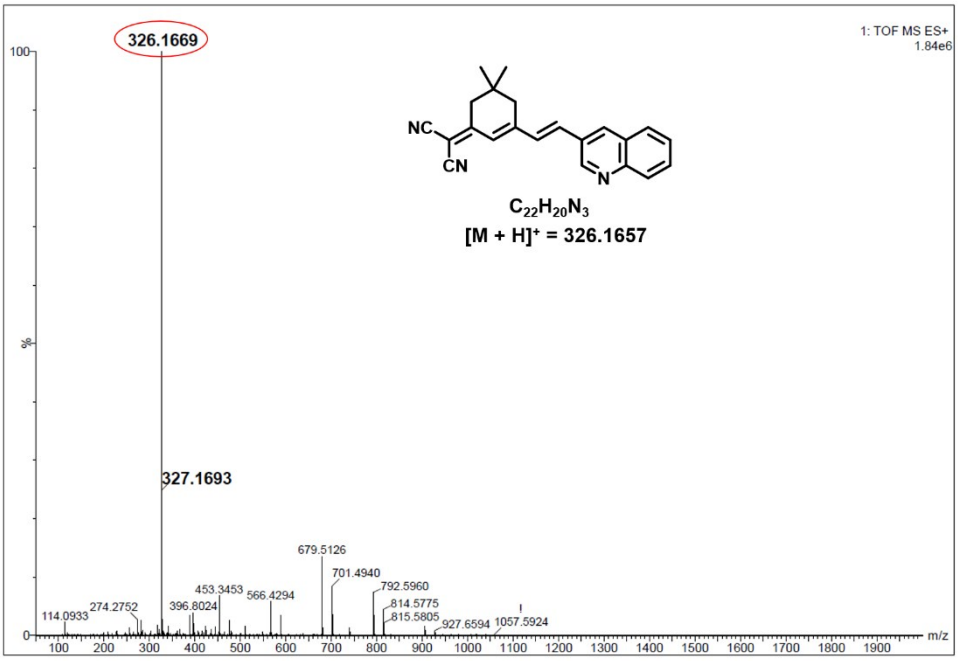

Fig. S10. HR-MS spectrum of **DCIQ**.

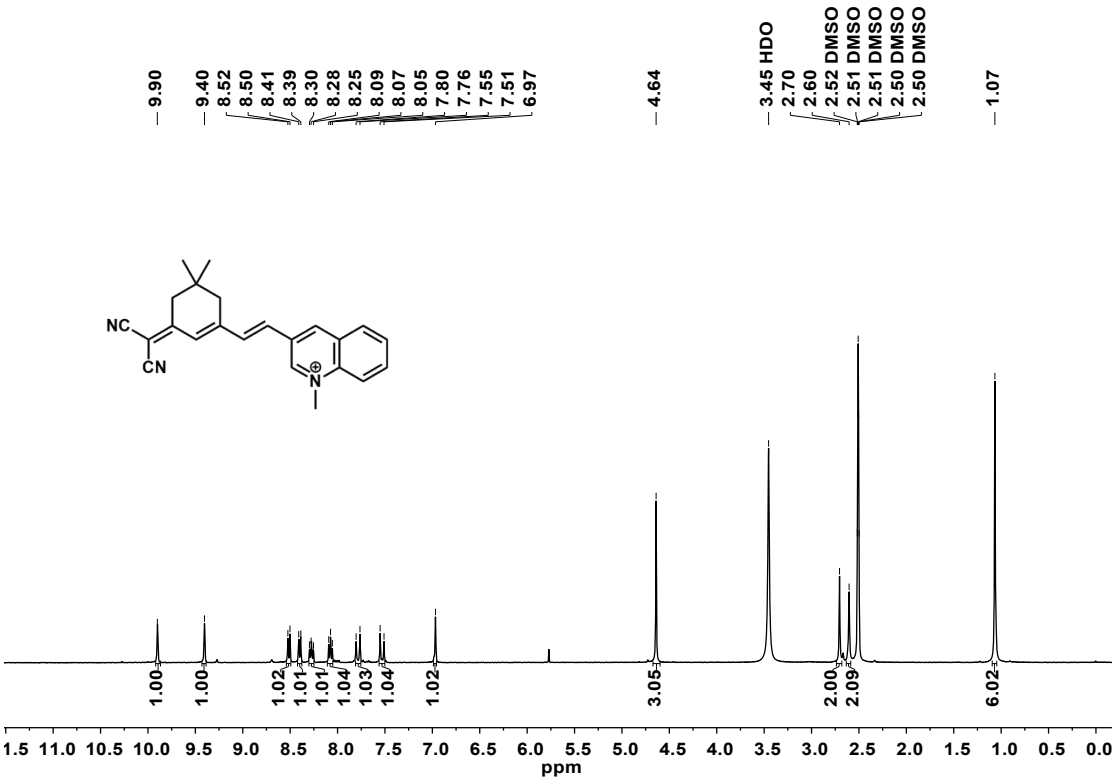

Fig. S11. ^{1}H NMR spectrum of **DCQN** in DMSO-d_6 (400 MHz).

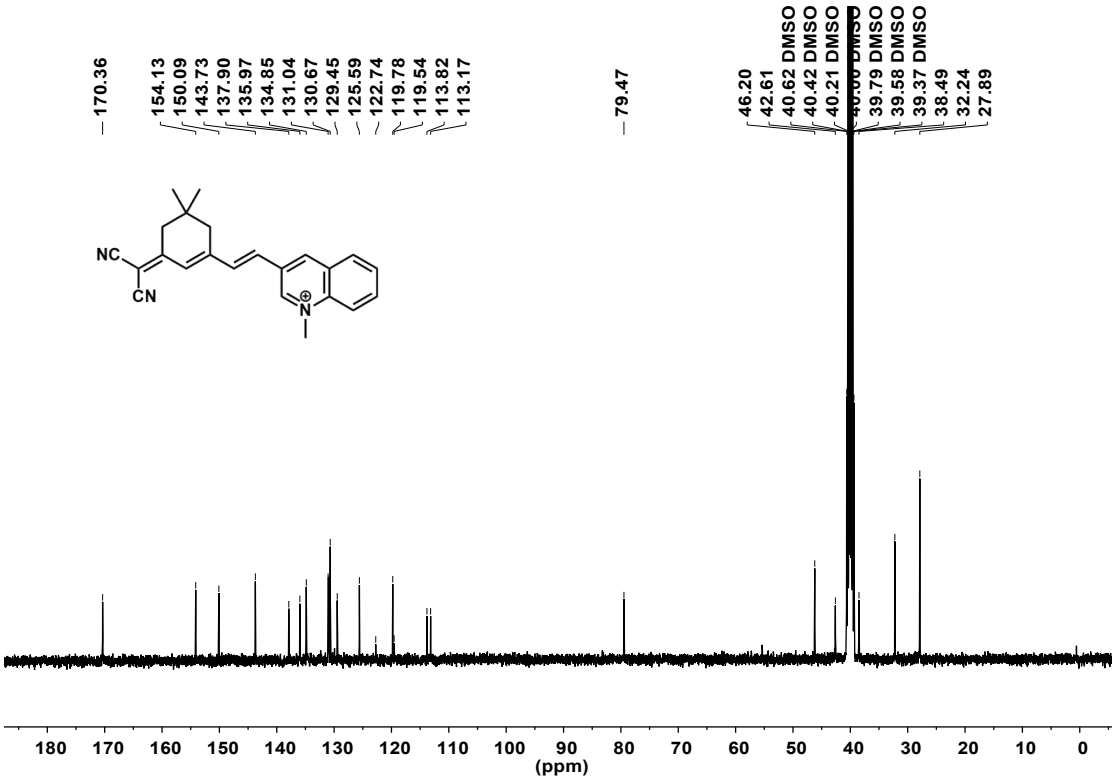

Fig. S12.[13]C NMR spectrum of **DCQN** in DMSO-d_6 (100 MHz).

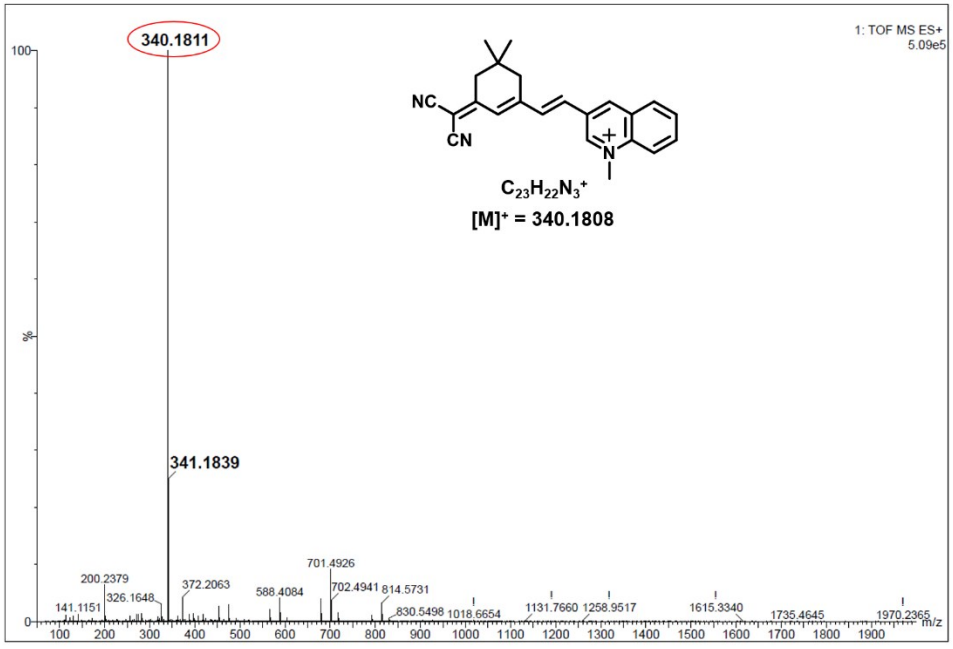

Fig. S13. HR-MS spectrum of **DCQN**.

References

1. K. Umezawa, A. Matsui, Y. Nakamura, D. Citterio, K. Suzuki, Chem.-Eur. J., 2009, 15, 1096-1106.