Supporting Information

Water solubility is essential for fluorescent probes to image hypochlorous acid in live cells

Panfei Xing,[‡a] Zhe Zhang,[‡a] Yiming Niu,[a] Yaning Qi,[b] Lei Dong*[c] and Chunming Wang*[a]

Table of Contents

Experimental section

Materials and Instruments. All the solvents and chemical reagents were commercial source, analytical grade and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 MHz AVANCE III spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were obtained with Thermo Scientific MSQ Plus mass spectrometer (USA). Absorption spectra were collected by using HACH DR6000 UV/VIS Spectrophotometer (USA). Fluorescence spectra were measured with a Horiba Fluorolog-3 spectrofluorometer. The fluorescence imaging of cells was performed using a Leica SP8 TCS confocal laser scanning microscope.

NDMTC was synthesized according to our reported paper.[1] And QCy 7 derivatives and 4-Methylumbelliferone was synthesized according to the reported literature.[2]

4-Methylumbelliferone. Concentrated phosphoric acid (10 mL, 85%) was added into a mixture of 3-hydroxylphenol (1.1 g, 10 mmol) and ethyl acetoacetate (1.3 mL, 10 mmol). Then the mixture was stirred for 12 h at room temperature. After the reaction complete, the mixture was poured into 30 mL water. The crude product was filtered and purified on silica gel (dichloromethane/petroleum ether = 1:1) to afford a white solid with a yield of 79%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58 (d, J = 8.7 Hz, 1H), 6.79 (dd, J = 8.7 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.11 (dd, J = 8.4 Hz, 1H), 2.35 (d, 3H).

Synthesis of CAMTC. A mixture of 4-Methylumbelliferone (176 mg, 1.0 mmol) and N,N-Diisopropylethylamine (150 μL) was dissolved in dichloromethane (30 mL), stirring for 15 mins. Then, dimethylcarbamothioic chloride (250 mg, 2.0 mmol) was added and the mixture was stirred for another 6 h. The crude product was purified on silica gel (dichloromethane/petroleum ether = 1:1) to afford a white solid with a yield of 63%.^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, 1H), 7.05 (dd, J = 6.8 Hz, 2H), 6.26 (s, 1H), 3.46 (s, 3H), 3.37 (s, 3H), 2.43 (d, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.70, 160.58, 156.20, 154.06, 152.06, 124.99, 119.64, 117.93, 114.51, 111.77, 43.39, 38.97, 18.78. m/z: $[M + Na]^+$ Calcd. for $C_{13}H_{13}NNaO_3S^+$ 286.0508, Found 286.0513.

Synthesis of QYMTC and **QEMTC.** QCy 7 derivatives (1.0 mmol) and and N,N-Diisopropylethylamine (150 μL) was dissolved in anhydrous acetonitrile (20 mL), stirring for 20 mins. Then, dimethylcarbamothioic chloride (250 mg, 2.0 mmol) was added and the mixture was stirred for another 20 h. The crude product was purified on silica gel (dichloromethane/methanol = 5:1) to afford a red solid with a yield of 41% and 39%, respectively. **QYMTC.** ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.32 (d, J = 8.3 Hz, 1H), 8.61 (dd, J = 8.2 Hz, 2H), 8.45 (d, J = 8.2 Hz, 2H), 8.20-8.13 (m, 1H), 8.09 (t, J = 8.1 Hz, 1H), 7.86 (dd, J = 8.1 Hz, 2H), 7.71-7.58 (m, 4H), 5.04-4.96 (m, 2H), 3.22-3.14 (m, 2H), 2.93 (s, 2H), 2.69 (dd, 4H), 2.44-2.38 (m, 2H), 2.30-2.18 (m, 2H), 1.86 (s, 6H), 1.73 (s, 6H), 1.15 (t, 3H), 1.05 (t, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.28, 181.92, 163.03, 151.55, 149.46, 144.43, 143.97, 140.92, 140.80, 138.37, 137.69, 136.61, 135.87, 132.67, 131.71, 129.95, 129.88, 129.31, 129.16, 123.13, 115.84, 115.65, 115.38, 68.96, 65.06, 52.72, 52.21, 48.42, 47.09, 46.99, 45.95, 45.52, 34.24, 25.79, 25.72, 25.58, 15.17, 8.50. m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{45}N_3NaO_7S_3^+$ 786.2312, Found 786.2381. **QEMTC.** ^{1}H NMR (600 MHz, DMSO-d_6)

δ 9.47 (s, 1H), 8.81 (d, *J* = 8.4 Hz, 1H), 8.68 (dd, *J* = 8.2 Hz, 1H), 8.60 (dd, *J* = 8.4 Hz, 1H), 8.51-8.45 (m, 1H), 8.38-8.34 (m, 2H), 8.33 (d, *J* = 7.9 Hz, 1H), 8.30 (dd, *J* = 9.0 Hz, 1H), 8.25 (dd, *J* = 8.5 Hz, 2H), 5.11 (dd, 2H), 2.88 (dd, 4H), 2.85 (d, 2H), 2.78-2.71 (m, 2H), 2.40-2.30 (dd, 4H), 2.14 (s, 6H), 2.00 (s, 6H), 1.18 (t, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 185.63, 183.44, 182.63, 156.76, 151.48, 143.82, 139.80, 139.66, 139.06, 138.95, 135.06, 133.93, 133.84, 132.18, 131.73, 131.60, 130.62, 130.54, 129.30, 128.95, 128.03, 127.87, 127.19, 125.74, 123.89, 123.46, 115.29, 114.23, 113.94, 54.59, 54.21, 47.67, 47.63, 43.57, 34.53, 26.24, 26.16, 26.11, 26.06, 25.55, 25.48. m/z: $[M + Na]^+$ Calcd. for $C_{47}H_{49}N_3NaO_7S_3^+$ 886.2625, Found 886.2634.

The calculation of LOD

The fluorescence intensity of ten reagent blank samples containing no HClO was measured, and the mean as well as the standard deviation (SD) was calculated.

$$LOD = 3\sigma/k$$

Where 3 is used for signal to noise ratio, σ is the SD of blank measurement and k is the slope of the calibration curve

Cell Culture

RAW 264.7 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO2. Then cells were transferred to culture dishes. Cell imaging was carried out after washing the cells with PBS (10 mM, pH 7.4).

Supplemental figures

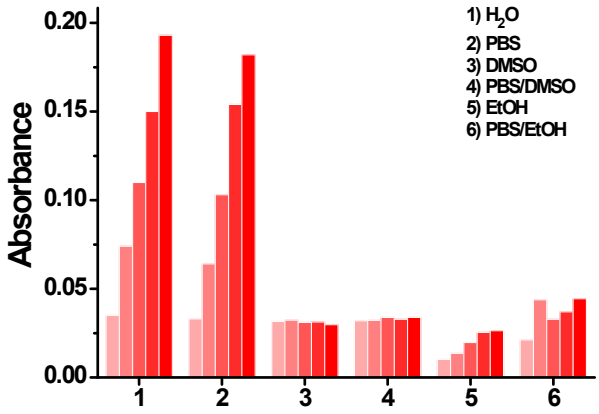

Fig. S1 Concentration-dependent absorption of different equivalent of NaClO (1-5 equiv.) in different solutions. The value was collected at the maximum absorption. 1, H_2O; 2, PBS (10 mM, pH 7.4); 3, DMSO; 4, PBS/DMSO (1:1, v/v); 5, EtOH; 6, PBS/EtOH (1:1, v/v).

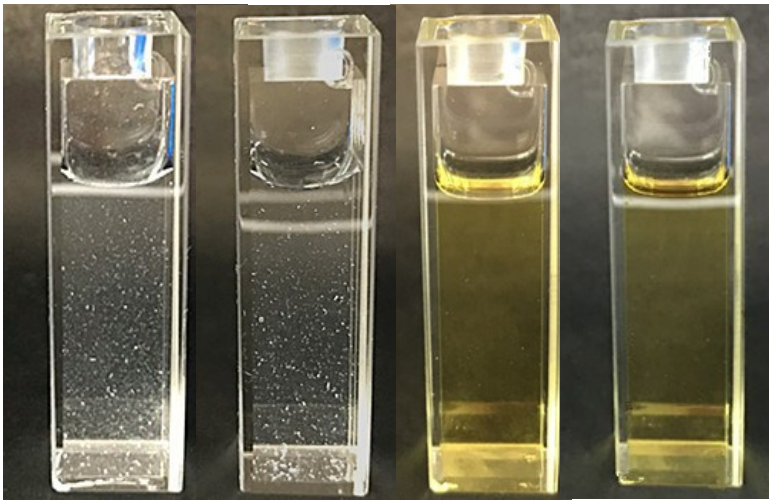

Fig. S2 Photos of **CAMTC**, **NDMTC**, **QYMTC**, and **QEMTC** (20 μM for each) in PBS (10 mM, pH 7.4) solution.

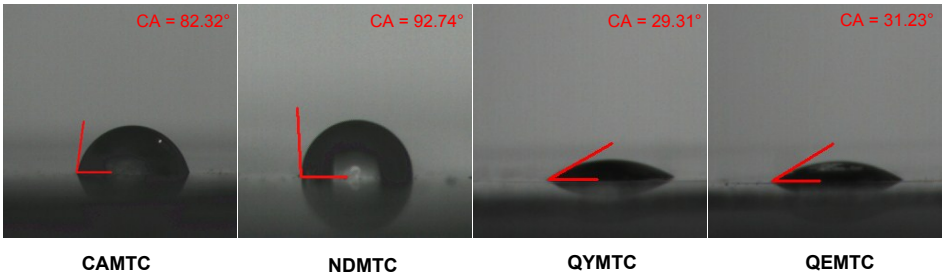

Fig. S3 Contact angle (CA) **CAMTC**, **NDMTC**, **QYMTC**, and **QEMTC**.

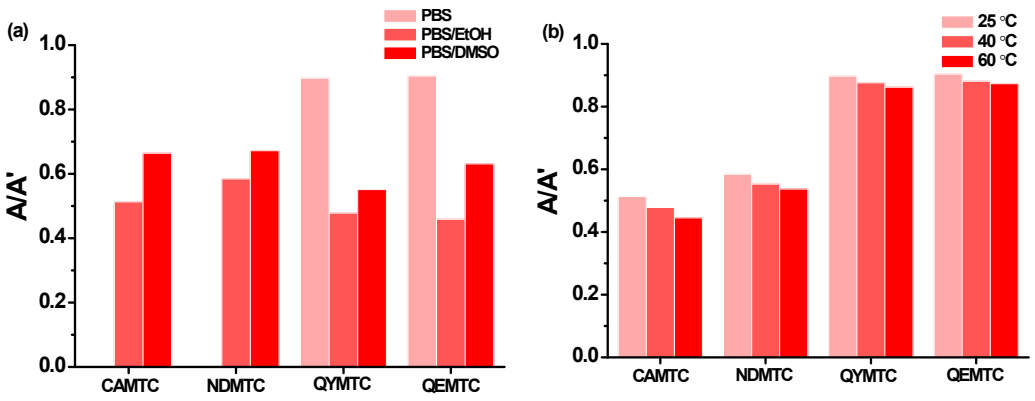

Fig. S4 The solvent and temperature effect on the absorption of probes in the presence of NaClO. a) Absorption response of **CAMTC**, **NDMTC**, **QYMTC** and **QEMTC** (10 μM for each) in the presence of NaClO (150 μM) in different solutions (PBS; PBS/EtOH, 1:1, v/v; PBS/DMSO, 1:1, v/v); b) **CAMTC** and **NDMTC** were prepared in PBS/EtOH (1:1, v/v); **QYMTC** and **QEMTC** were prepared in PBS (10 mM, pH 7.4). A and A' represent the maximum absorbance value with and without the addition of NaClO, respectively.

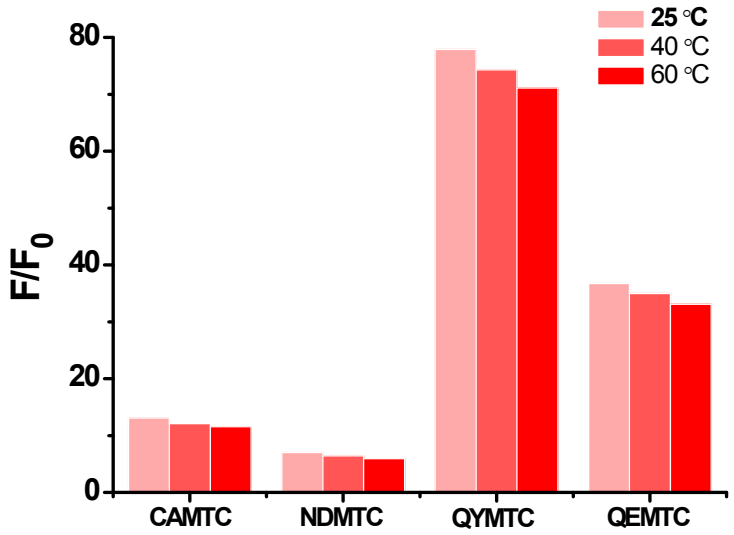

Fig. S5 The temperature effect on the fluorescence of probes in the presence of NaClO. **CAMTC** and **NDMTC** were prepared in PBS/EtOH (1:1, v/v); **QYMTC** and **QEMTC** were prepared in PBS (10 mM, pH 7.4).

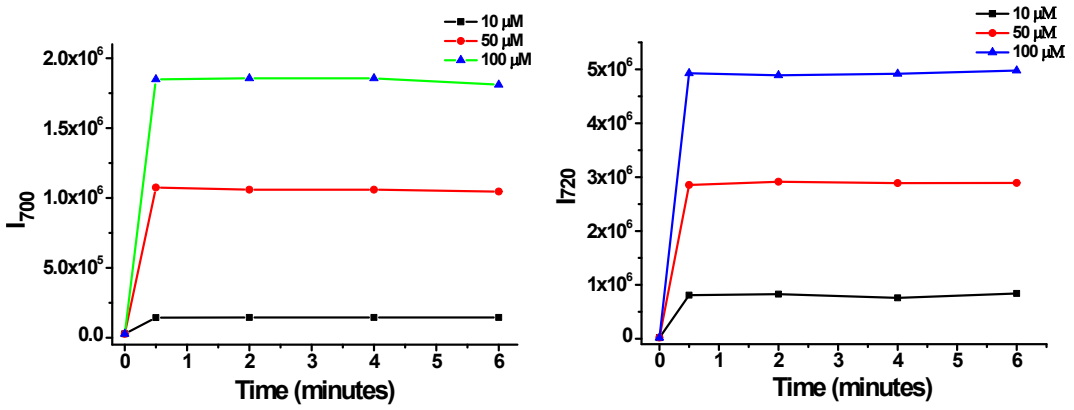

Fig. S6 Plot of fluorescence intensity versus time for the reaction of **QYMTC** (10 μM) and **QEMTC** (10 μM) with NaClO in PBS (10 mM, pH 7.4).

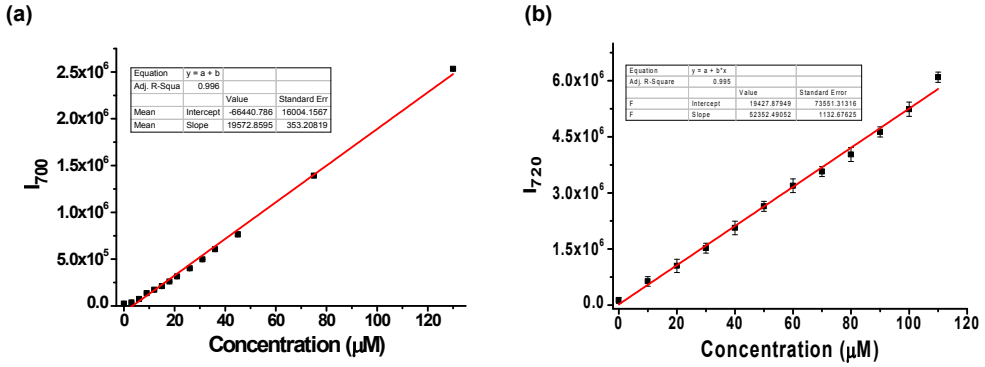

Fig. S7 Linear relationship between different concentration of HClO and the emission of probe **QYMTC** (10 μM, a) and **QEMTC** (10 μM, b) in PBS (10 mM, pH 7.4).

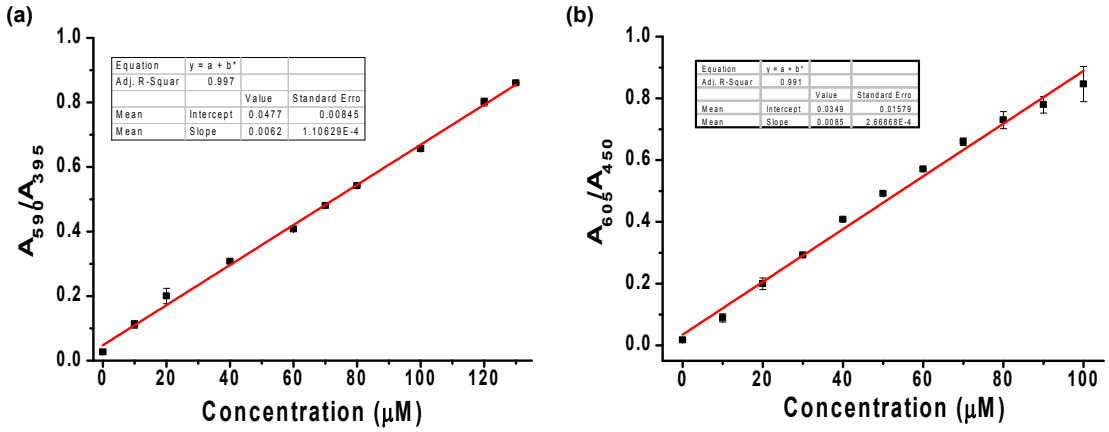

Fig. S8 Linear relationship between different concentration of HClO and the absorption of probe **QYMTC** (10 μM, a) and **QEMTC** (10 μM, b) in PBS (10 mM, pH 7.4).

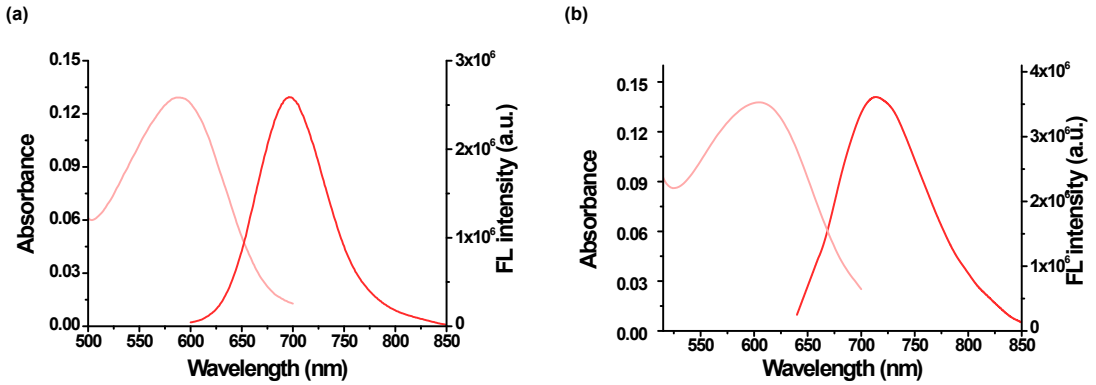

Fig. S9 Absorption and emission spectrum of **QYMTC** (10 μM, a) and **QEMTC** (10 μM, b) upon the addition of saturated HClO in PBS (10 mM, pH 7.4).

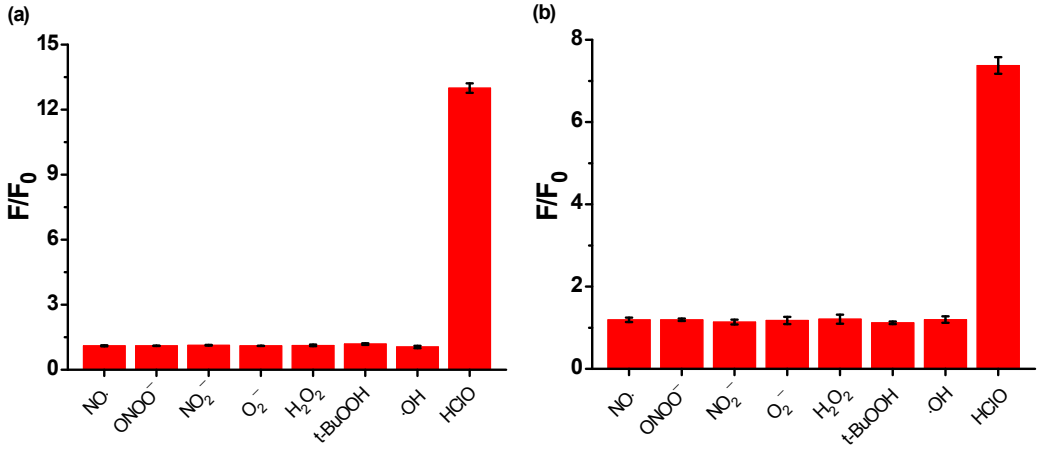

Fig. S10 Competitive experiments for HClO selectivity. Fluorescence responses of **CAMTC** (10 μM) and **NDMTC** (10 μM) to HClO (150 μM) and other ROS/RNS (200 μM for each) in PBS/EtOH (10 mM, pH 7.4; v/v, 1:1) at room temperature.

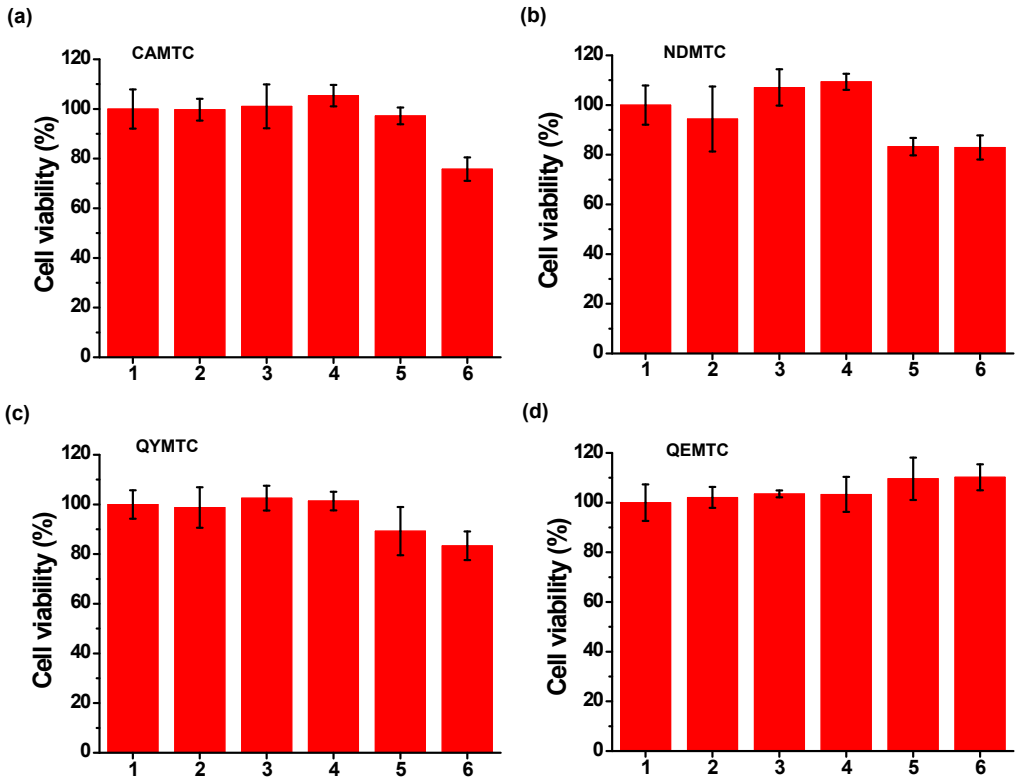

Fig. S11 The cytotoxicity of different concentrations of **CAMTC**, **NDMTC**, **QYMTC**, and **QEMTC** (0, 5, 10, 20, 30, 40 and 50 μM) in RAW 264.7 cells.

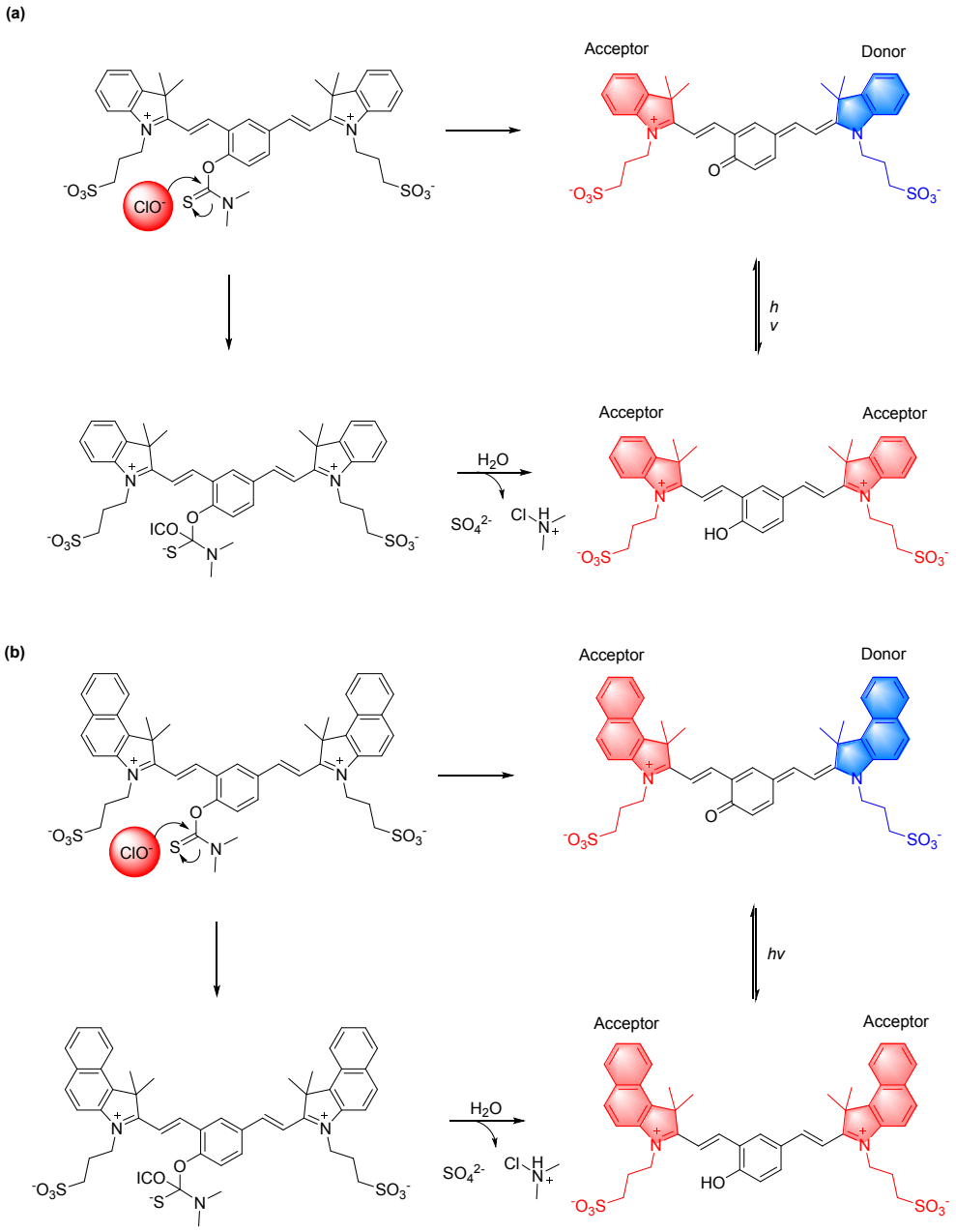

Fig. S12 Proposed mechanism for the HClO detection for **QYMTC** and **QEMTC**.

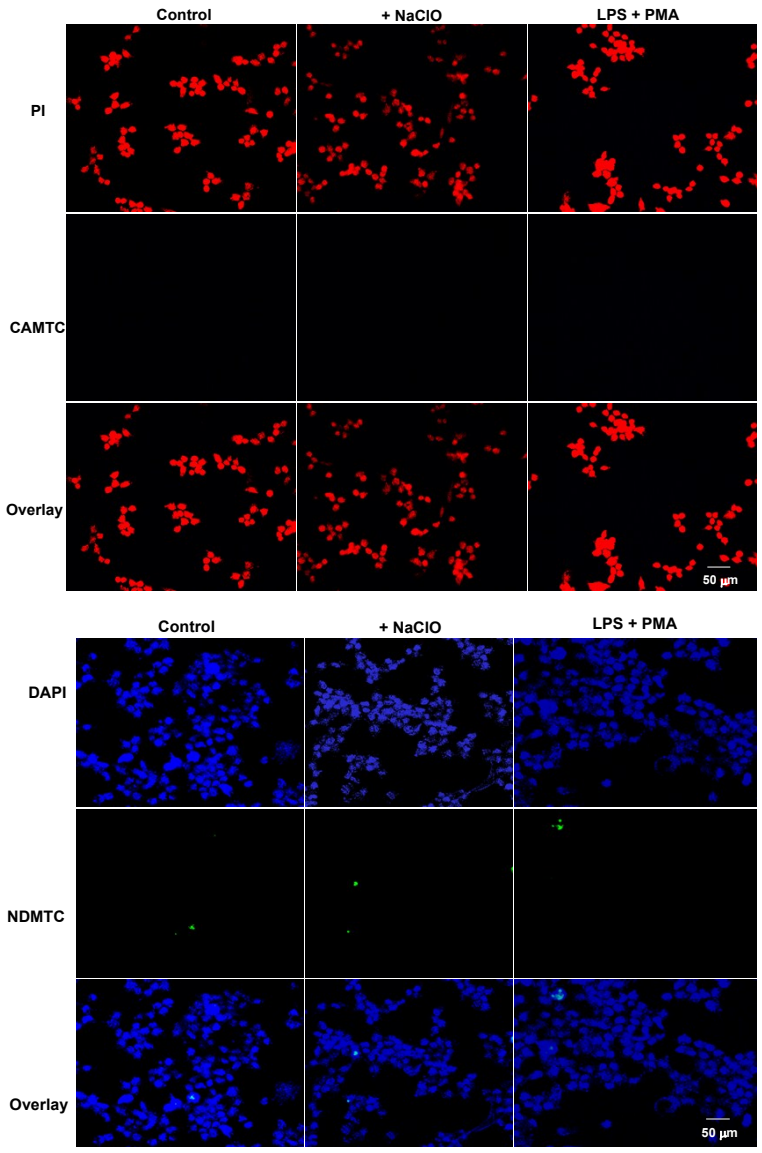

Fig. S13 Confocal imaging of **CAMTC** and **NDMTC** in the detection of exogenous and endogenous HClO. NaClO (20 μM) to generate exogenous HClO, LPS (0.5 μg/mL) plus PMA (1 μg/mL) as an established protocol to induce endogenous HClO, and the cells received no further treatment as control group. Then cells were incubated with **CAMTC** (10 μM, as a) or **NDMTC** 10 μM, as b) for 20 mins, fixed with formaldehyde, and stained with PI (100 ng mL^{-1}, for **CAMTC**) or DAPI (100 ng mL^{-1}, for **NDMTC**) for another 10 mins. Scale bar was 50 μm. The excitation wavelength for **CAMTC** was 405 nm and the emissions were collected at 430–520 nm; The excitation wavelength for **NDMTC** was 488 nm and the emissions were collected at 520-580 nm; The excitation wavelength for **DAPI** was 405 nm and the emissions were collected at 430-460 nm; The excitation wavelength for **PI** was 535 nm and the emissions were collected at 550-650 nm.

Characterization

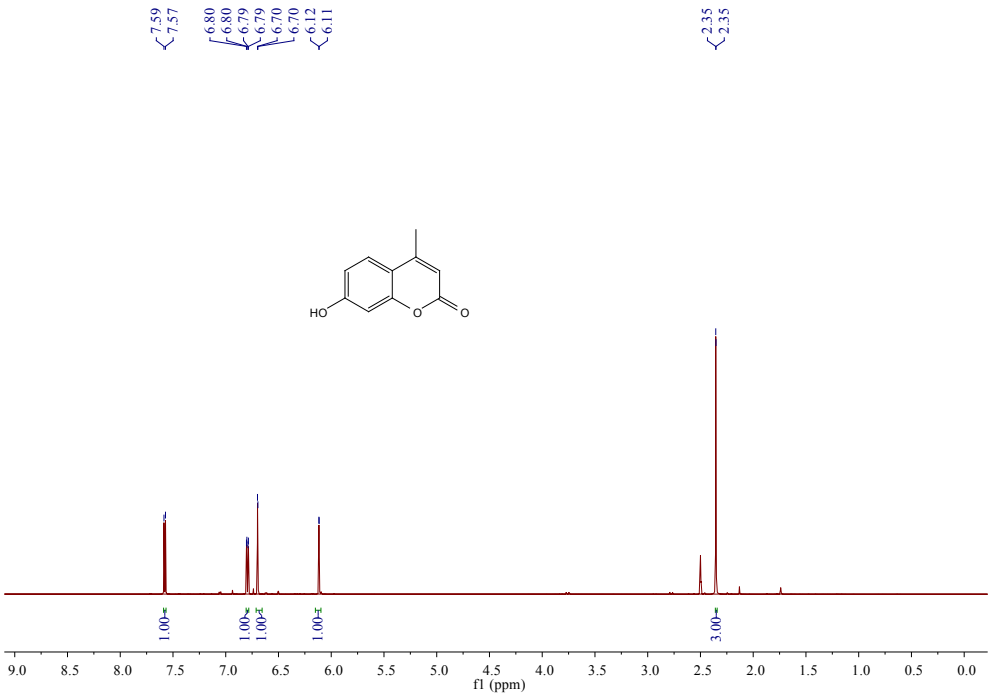

Fig. S14 ^{1}H NMR of 4-Methylumbelliferone in DMSO-d_6.

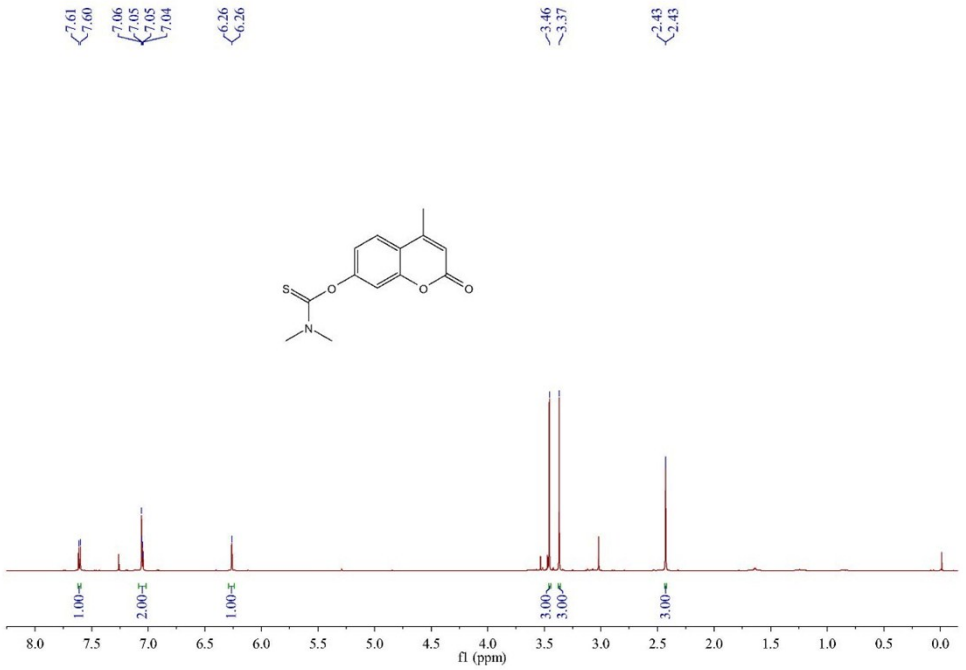

Fig. S15 ^{1}H NMR of **CAMTC** in $CDCl_3$.

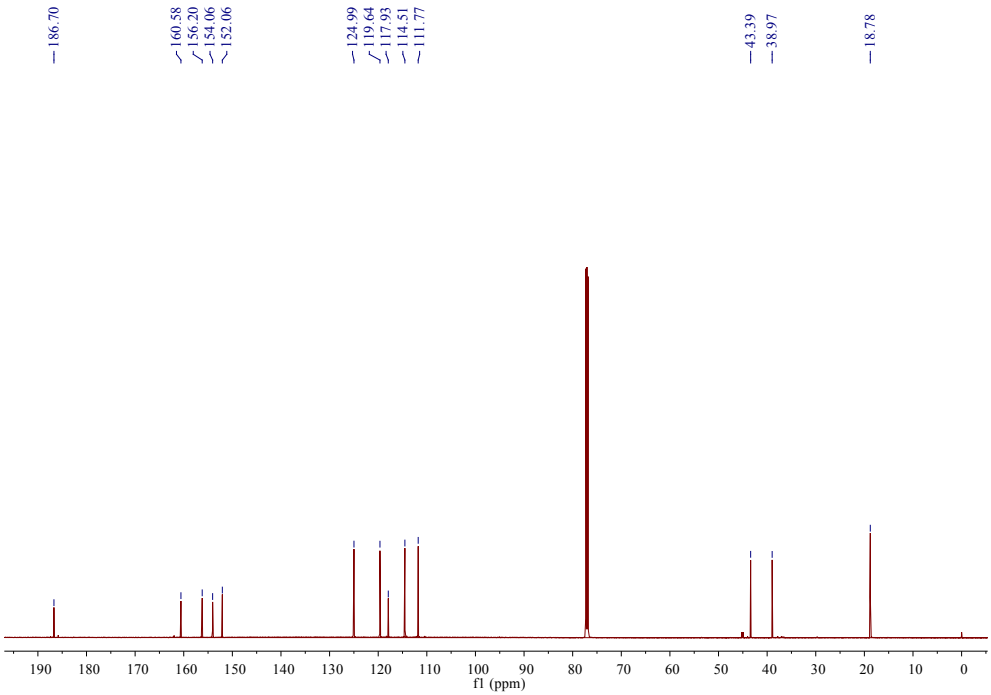

Fig. S16 ^{13}C NMR of **CAMTC** in $CDCl_3$.

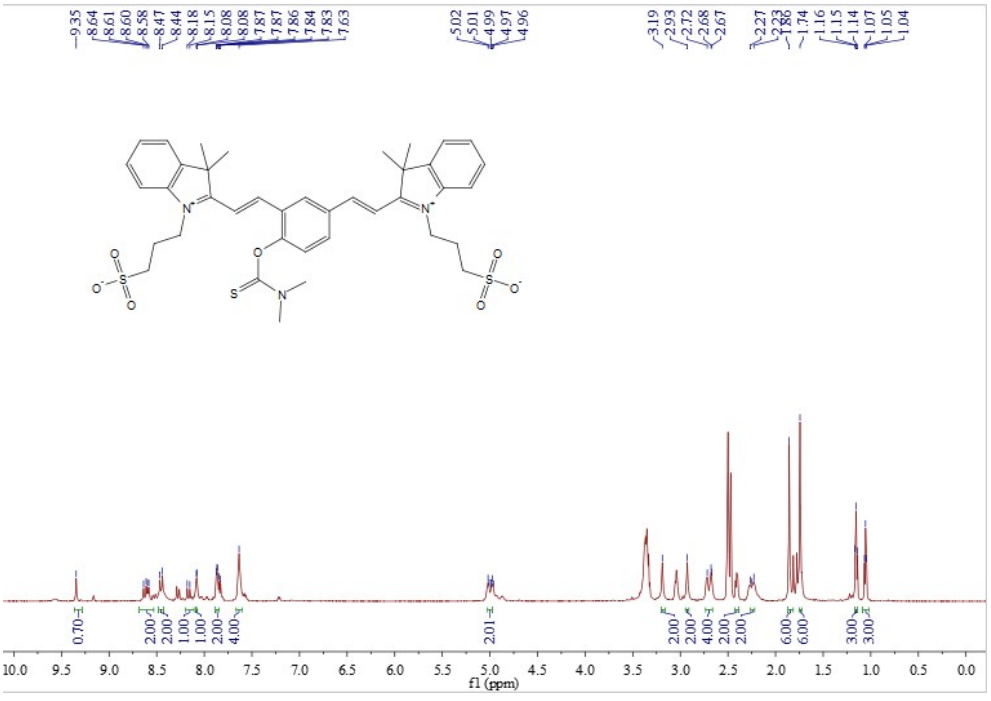

Fig. S17 ^{1}H NMR of **QYMTC** in DMSO-d_6.

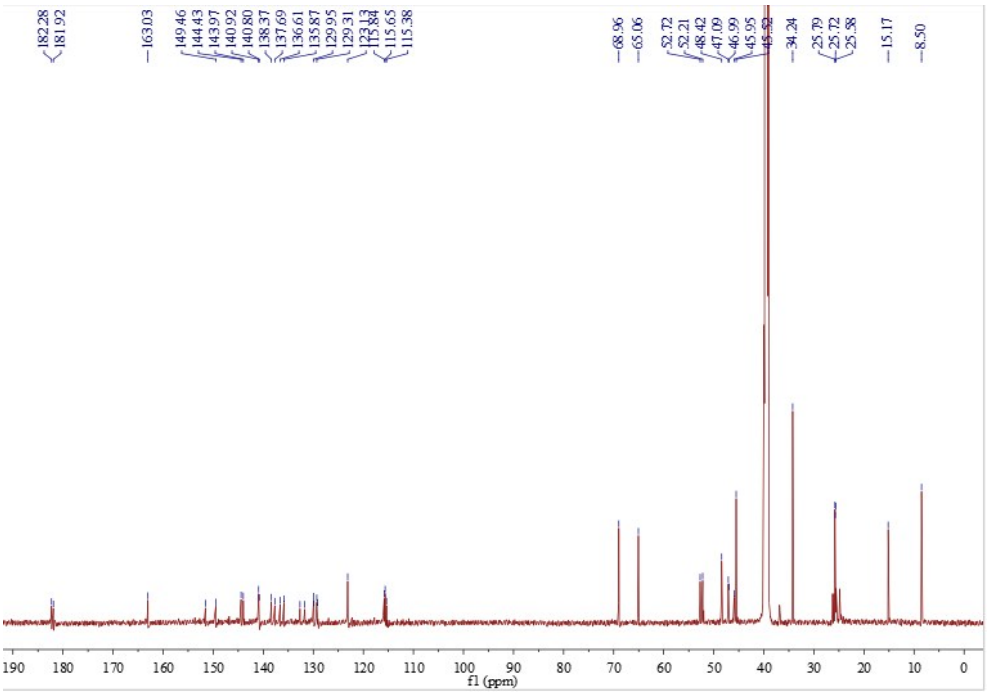

Fig. S18 ^{13}C NMR of **QYMTC** in DMSO-d_6.

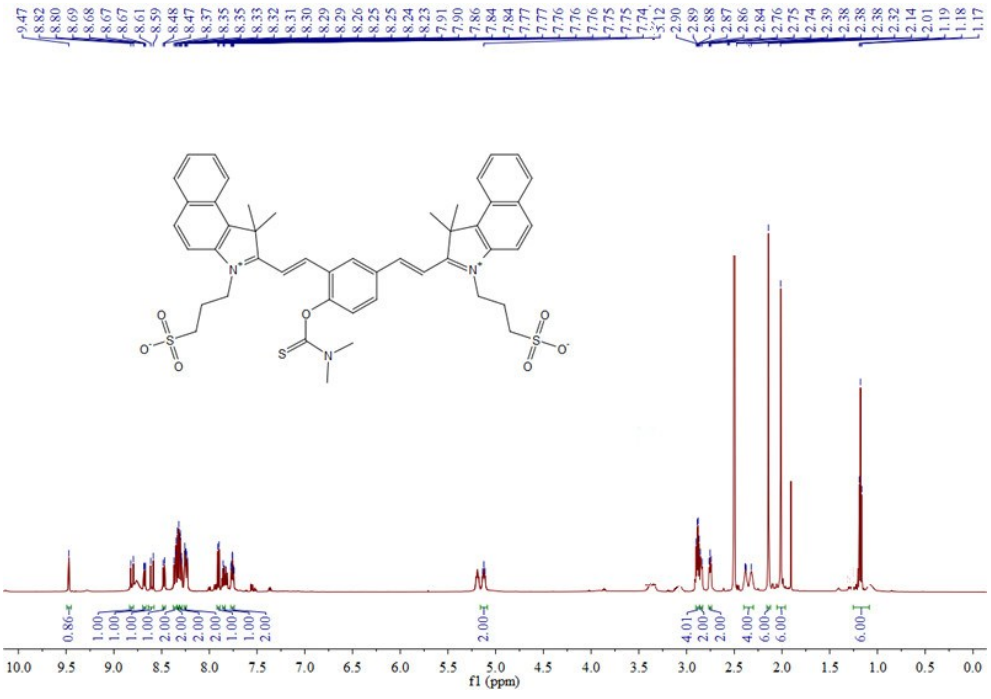

Fig. S19 ^{1}H NMR of **QEMTC** in DMSO-d_6.

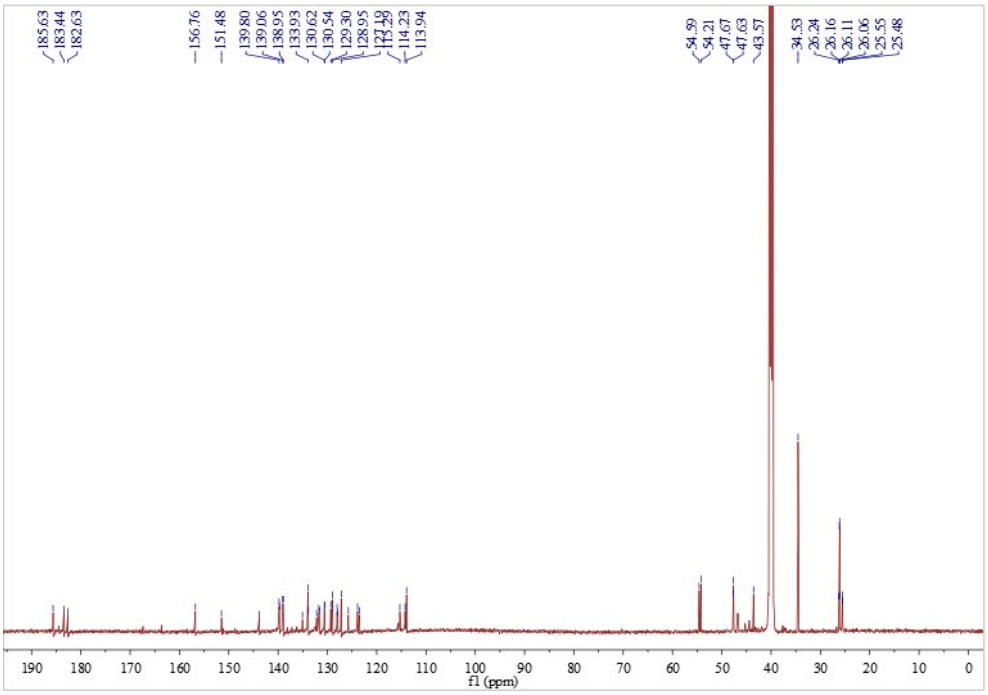

Fig. S20 ^{13}C NMR of **QEMTC** in DMSO-d_6.

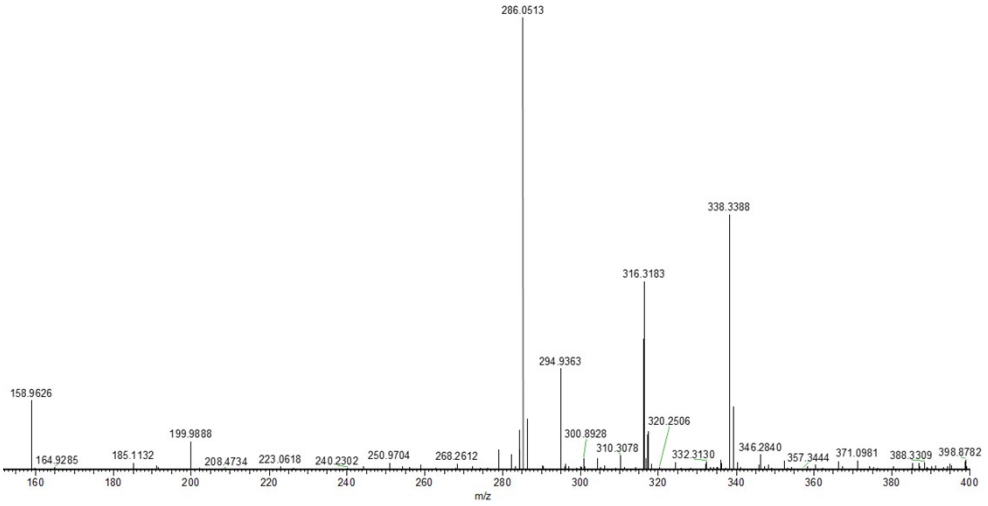

Fig. S21 HRMS of **CAMTC** $[M+Na]^+$.

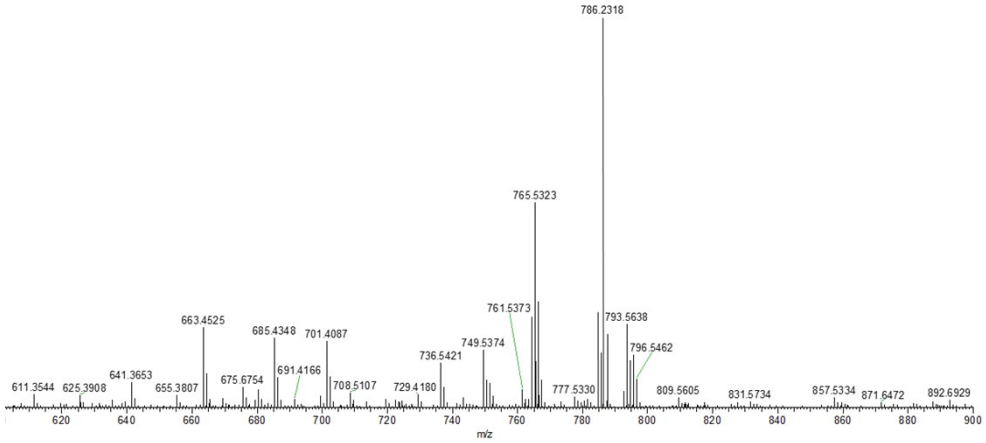

Fig. S22 HRMS of **QYMTC** $[M+Na]^+$.

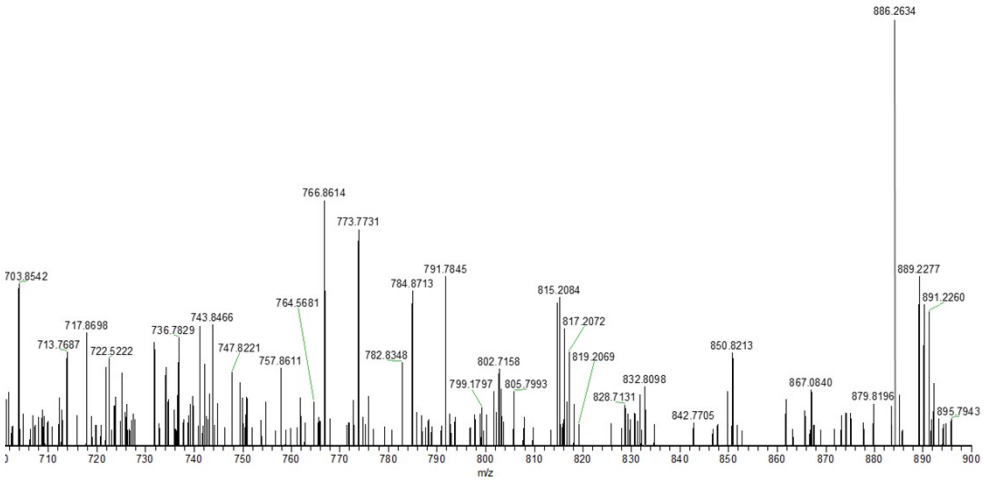

Fig. S23 HRMS of **QEMTC** $[M+Na]^+$.

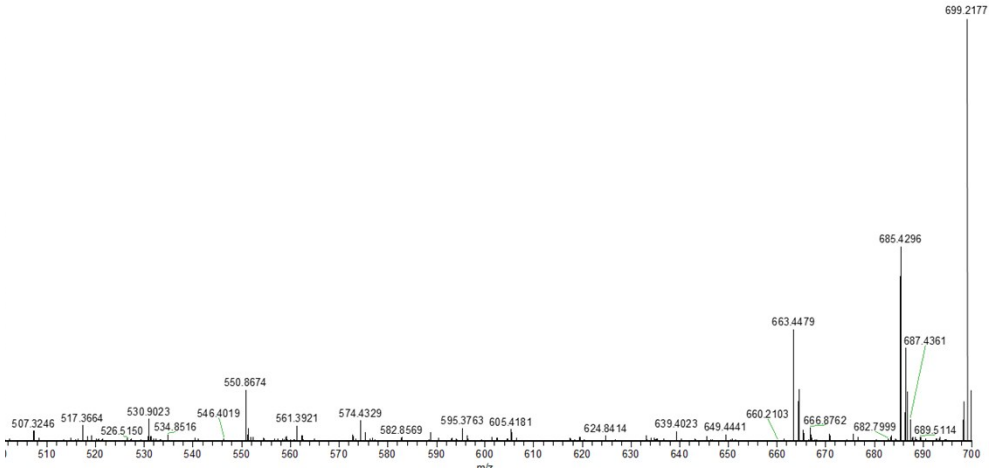

Fig. S24 HRMS of **QYMTC** with the addition of NaClO $[M+Na]^+$.

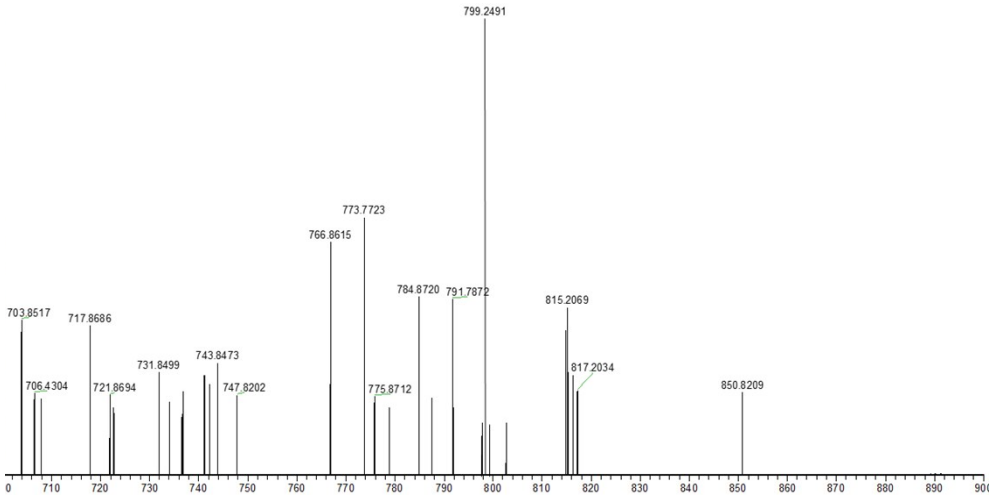

Fig. S25 HRMS of **QEMTC** with the addition of NaClO $[M+Na]^+$.

Reference

1. P. Xing, Y. Feng, Y. Niu, Q. Li, Z. Zhang, L. Dong and C. Wang, *Chem.-Eur. J.*, 2018, **24**, 5748-5453.
2. (a) Y.-F. Kang, H.-X. Qiao, Y.-L. Meng, S.-J. Cui, Y.-J. Han, Z.-Y. Wu, J. Wu, X.-H. Jia, X.-L. Zhang and M.-Y. Dai, *RSC Adv.*, 2016, **6**, 94866-94869; (b) O. Redy-Keisar, E. Kisin-Finfer, S. Ferber, R. Satchi-Fainaro and D. Shabat, *Nat. Protoc.*, 2014, **9**, 27-36.