Electronic Supplementary Information for

A Lysosome-Targeting and Polarity-Specific Fluorescent Probe for Cancer Diagnosis

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Experimental section

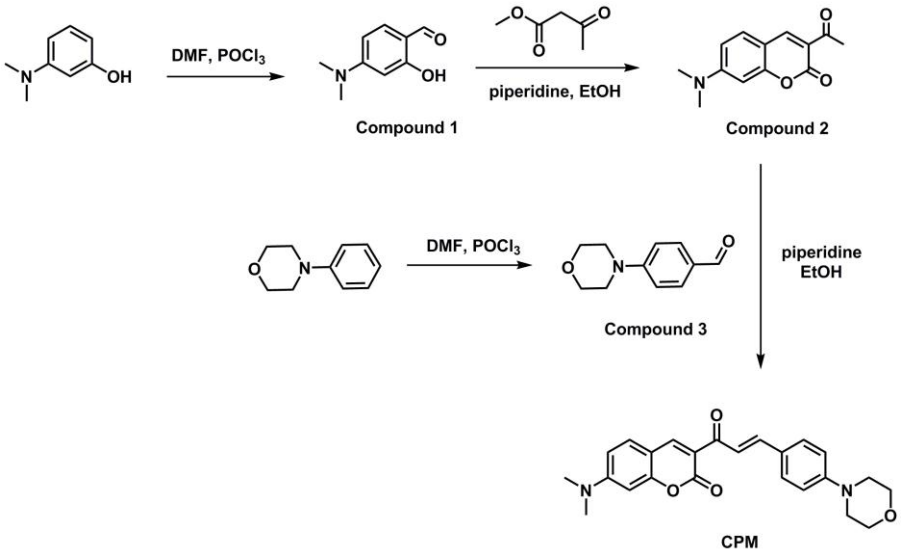
Materials and apparatus

Phosphorus oxychloride, 3-(dimethylamino)phenol, ethyl acetoac, 4-phenylmorpholine and lipopolysaccharides (LPS) were purchased from Sigma-Aldrich company. LysoTracker Green DND-26 was commercially available from Invitrogen (USA). All other chemicals and solvents with spectroscopical pure were bought from Tianjin chemical reagent company.

NMR spectra were recorded on Varian NMR System 300 MHz (Mecury, Varian, Inc., USA) using tetramethylsilane (TMS) as the internal standard in the solvent deuterated deuterated chloroform ($CDCl_3$) with 300 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively. HR-MS was measured by an Agilent Accurate-Mass-Q-TOF MS 6520 system equipped with an electrospray ionization (ESI) source (Agilent, USA). Deionized water was obtained with a Milli-Q water purification system (Millipore). The pH value was performed on a Beckman pH-3c digital meter (Shanghai LeiCi Device Works, Shanghai, China). The UV-visible spectra were taken on a TU-1901 double-beam UV-vis spectrophotometer (Beijing Purkinje General Instrument Co., LTD, Beijing, China). Fluorescence spectra were carried out on a FLS-920 Edinburgh Fluorescence Spectrophotometer (Edinburgh Co., Ltd., England) equipped with a xenon discharge lamp using 1 ml Fluor Micro Cell. Live cells and tissue fluorescence images were taken on a confocal laser scanning microscope (Zeiss, LSM880, Germany) with red channel (Ex = 458 nm, Em = 560 - 650 nm) for **CPM**. *In vivo* images were then taken by using a Bruker small animal *in vivo* Xtreme imaging system, with an excitation filter of 470 nm and an emission filter of 600 nm.

Synthesis and characterization

Synthesis routine of CPM



Scheme **S1** The synthetic routine of **CPM**

Synthesis of Compound 1. 7 mL phosphorus oxychloride (75 mmol) was added dropwise to the solution of 3-(dimethylamino)phenol (5.00 g, 36.5 mmol) in 15 mL dry DMF at 0 ℃ over 5 min, then the reaction mixture was stirred for 30 min at room temperature, followed for 1 h at 65 ℃. The solution was poured into 70 mL ice water, and the product was extracted with ethyl acetate three times and saturated sodium chloride aqueous solution one time. The organic layer was dried over anhydrous magnesium sulfate filtered and evaporated, affording compound **1** as a white powder 4.00 g (66 % yield). The crude product compound **1** was used for the subsequent synthesis without further purification and characterization.

Synthesis of Compound 2. A mixture of 1.00 g (6.05 mmol) compound **1**, 0.99 g (8.53 mmol) ethyl acetoac and 0.50 ml piperidine was refluxed in 12 mL absolute ethanol for 3h. The reaction was cooled to room temperature. Then the precipitated solid was concentrated under vacuum and washed with absolute ethanol, affording compound **2** as a yellow powder 1.30 g (92 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.44 (s, 1H, Ar-H), 7.42-7.24 (d, 1H, Ar-H), 6.65-6.61 (m, 1H, Ar-H), 6.46-6.45 (m, 1H, Ar-H), 3.11 (s, 6H, -CH_3), 2.60 (s, 3H, -CH_3).

Synthesis of Compound 3. 3.4 mL phosphorus oxychloride (36 mmol) was added

dropwise to the solution of 4-phenylmorpholine (3.00 g, 18.4 mmol) in 10 mL dry DMF at 0 ℃ over 5 min, then the reaction mixture was stirred for 30 min at room temperature, followed for 1 h at 65℃. The solution was poured into 40 mL ice water, and the product was extracted with ethyl acetate three times and saturated sodium chloride aqueous solution one time. The organic layer was dried over anhydrous magnesium sulfate filtered and evaporated. The residue was purified by chromatography on a silica gel column using ethyl acetate/hexane (5/1, V/V) as the eluent, affording compound **3** as a white powder (2.80 g, 80 % yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 9.80 (s, 1H, -CHO), 7.78-7.75 (d, 2H, Ar-H), 6.94-6.91 (d, 2H, Ar-H), 3.87-3.84 (m, 4H, -CH_2-), 3.36-3.33 (m, 4H, -CH_2-).

Synthesis of CPM. A mixture of 1.00 g (4.32 mmol) compound **2**, 0.83 g (4.44 mmol) compound **3** and 0.53 ml piperidine are stirred in 15 ml absolute ethanol at room temperature for 10 min, followed at 65 ℃ over night. Then the reaction was cooled to room temperature, and the precipitated solid was collected by filtration. Then the red power (1.50 g, 3.71 mmol) was obtained after the precipitate washed with absolute ethanol and dried. Yield: 86 %. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.57 (s, 1H, Ar-H), 8.05-8.00 (d, 1H, Ar-H), 7.83-7.78 (d, 1H, Ar-H), 7.65-7.62 (m, 3H, Ar-H), 6.67-6.64 (m, 3H, Ar-H), 6.51-6.50 (m, 1H, Ar-H), 3.93-390 (m, 4H, -CH_2-), 3.31-3.29 (m, 4H, -CH_2-), 3.13 (s, 3H, -CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 186.36, 160.87, 158.17, 154.71, 152.60, 148.56, 143.82, 131.38, 130.48, 126.46, 121.52, 114.57, 109.94, 108.99, 97.10, 66.70, 48.06, 40.30. HR-MS m/z: $[M+H]^+$ calculated for $C_{24}H_{24}N_2O_4^+$, 405.1809; measured, 405.1805.

UV-vis and fluorescence measurements

A stock solution of **CPM** (1.0 mM) was prepared in DMSO. 10 μM of **CPM** was used in spectroscopic determination by addition of 20 μL stock solution to 2.0 mL different polarity solvents. Excitation and emission bandwidths were both set at 2.0 nm, and the excitation wavelength was fixed at 465 nm. All spectroscopic experiments were measured at room temperature. The relative fluorescence quantum yield of **CPM** in different polarity solvents was determined with rhodamine B (Φ_f =

0.89 in ethanol) as a fluorescence standard.

Cell culture and cell cytotoxicity assay

All the cells lines were kindly provided by Modern Research Center for Tradition Chinese (Shanxi University, Taiyuan, China). SMMC-7721, A549, HIC, HeLa, LO2, BEAS-2B and HIEC cells were cultured in RPMI1640 or DEME medium supplemented with 10 % fetal bovine serum and 1% antibiotics at 37 ℃ in a 5 % CO_2 atmosphere. The cell cytotoxicity of **CPM** to living SMMC-7721 cells was performed by a standard *CCK-8* assay (cell counting kit-8). About 1×10^4 cells/well in 200 μL cell culture medium were seeded in 96-well microplate and then the medium was replaced with fresh medium that containing **CPM** with various concentrations of 0 μM, 5 μM, 10 μM, 15 μM and 20 μM for 24 h, respectively. After washing the cells with fresh medium three times, 20 μL *CCK-8* in 180 μL PBS was loaded to each well for another 4 h. Then each well was analysed with an ELISA microplate reader and the absorbance was detected at 450 nm. The cell viability was expressed as relative to the control cells taken as 100 % metabolic activity.

Cell imaging and colocalization experiments

For live cell imaging, **CPM** was added to the cultured cells in a glass-bottomed dish for 10 min and washed with cold phosphate buffered saline (10 mM PBS, pH 7.4) three times. After replacement of the medium, cells were imaged using a confocal laser scanning microscope (Zeiss, LSM880) with red channel (Ex = 458 nm, Em = 560 - 650 nm), and a 63 × oil-immersion objective lens. For colocalization experiments, SMMC-7721 cells were plated on glass-bottomed dishes at a density of 1×10^5 for 24 h. **CPM** dissolved in DMSO (10 μL, 1 mM) were added to the cells medium (1 mL) at 5 μM or 3 μM final concentration for 10 min, then cells were washed with PBS (pH 7.4) three times. 1.0 μM LysoTracker Green DND-26, 0.2 μM MitoTracker Green or 1.0 μM ER-Tracker Green was then added and co-incubated for additional 30 min, respectively. After washed with PBS (pH 7.4) three times, fluorescence images of the cells were carried out on a confocal laser scanning microscope (Zeiss, LSM880) with a 63 × oil-immersion objective lens, and red

channel (Ex = 458 nm, Em = 560 - 650 nm) for **CPM**, Green channel (Ex = 488 nm, 500 – 550 nm) for Lyso Tracker DND-26, MitoTracker Green and ER-Tracker Green, respectively.

Preparation of living organs, tumor-bearing mice and *in vivo* imaging

KM mice (18-20 g) were purchased from Laboratory Animal Center of Shanxi Cancer Hospital (Taiyuan, China) for *in vivo* imaging. All animal experiments were performed in compliance with the Animal Management Rules of the Ministry of Health of the People's Republic of China (Document no. 55, 2001) and approved by the Animal Care and Use Committee of Shanxi University. We have taken great efforts to reduce the number of animal used in these studies and also taken effort to reduce animal suffering from pain and discomfort.

Tumor-bearing mice were prepared by subcutaneous injection of HeLa cells into the left axillae of mice over 10 days. For living organs imaging, the organs (heart, spleen, liver and kidney) and tumor were isolated from the mice. After washing by PBS (pH=7.4) for three times, these isolated organs and tumor were loaded with **CPM** (15 μM, 20 min), respectively, and finally subjected to *in vivo* imaging.

For living mice imaging, **CPM** (100 μL, 15 μM) was hypodermic injected into the tumour position of the tumor-bearing mice and the corresponding position of the normal mice, respectively. Before imaging, the mice were first anesthetized by an intraperitoneal injection of 4 % chloral hydrate (200 μL) for 5 min. Then, *in vivo* imaging were taken by using a Bruker small animal *in vivo* Xtreme imaging system, with an excitation filter of 470 nm and an emission filter of 600 nm.

Preparation of human normal tissue, human cancer tissue cryosections and fluorescence imaging

The harvested surgical specimens of patients, including human normal tissues (breast and thyroid) and their corresponding human cancer tissues (breast carcinoma in situ and thyroid microcarcinoma), were obtained from Taiyuan Central Hospital. Informed consent was obtained for any experimentation with human subjects. All the human tissues slices harvested from surgical specimens of patients and determined by doctors,

were cryosectioned as 5 μm thicknesses. The slices were incubated with **CPM** (5 μM) in PBS for 10 min, and then washed with PBS three times. The fluorescence images are carried out on a confocal laser scanning microscope (Zeiss, LSM880) with red channel (Ex = 458 nm, Em = 560 - 650 nm), and a 20 × water-immersion objective lens.

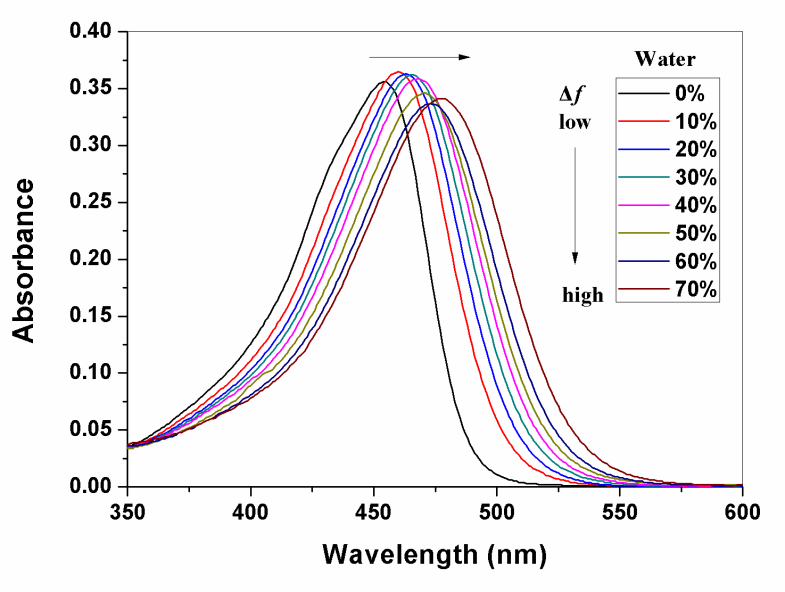


Fig. S1 Absorption spectra of **CPM** (10 μM) in different H_2O/THF solvent mixtures (water from 0% to 70%).

Table S1 Spectral properties of **CPM** in various solvents

Solvent	Dielectric Constant (ε)	Refractive Index (*n*)	Δf	λ_{abs} (nm)	λ_{em} (nm)	$\Delta\lambda$ (nm)	Extinction Coefficient (10^4 M^{-1} cm^{-1})	Φ_f (%)
Toluene	2.4	1.496	0.0153	453	550	97	4.046	25.09
Dioxane	2.21	1.4224	0.0205	450	565	115	3.597	10.53
Ethyl acetate	6.1	1.372	0.2012	459	602	143	2.506	9.85
THF	7.5	1.407	0.2087	454	605	151	4.381	8.46
Benzonitrile	25.9	1.5289	0.2356	4504	605	155	3.578	6.25
Acetonitrile	37.5	1.346	0.3055	473	608	135	3.123	2.25
Methanol	33.6	1.326	0.3101	458	550	152	3.428	1.28
Water	80.1	1.3333	0.3212	417	565	195	0.995	0.73

The dielectric constant data of the solvents were measured at 25 ℃. Solvent-dependent spectra are often interpreted in terms of the Lippert-Mataga equation.

$$f\,(\varepsilon) = (\varepsilon\text{-}1) / (2\varepsilon\text{+}1)$$

$$f\,(n^2) = (n^2\text{-}1) / (2n^2\text{+}1)$$

$$\Delta f = f\,(\varepsilon) - f\,(n^2)$$

Where ε and n are the dielectric constant and the refractive indices of the solvent, respectively.

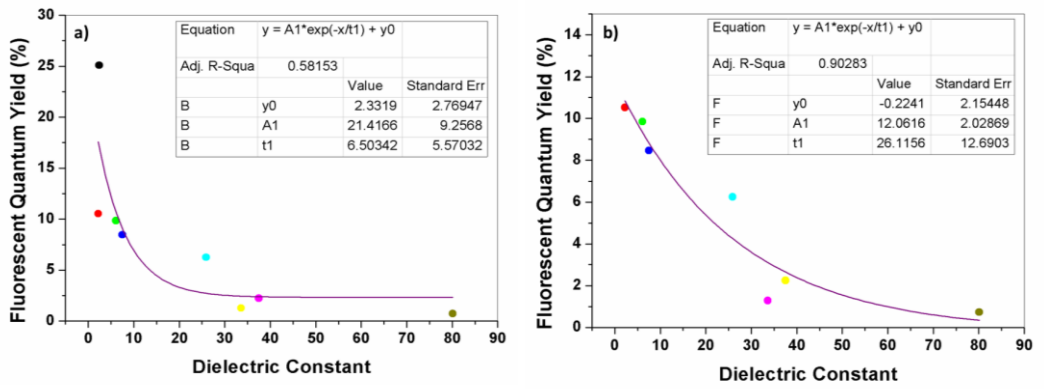


Fig. S2 (a) The 2D scatter fitting curve of fluorescence intensity of **CPM** versus dielectric constant in different solvents in Table S1, with a low relationship coefficient of R^2 = 0.58153. (b) The 2D scatter fitting curve of fluorescence intensity of **CPM** versus dielectric constant in different solvents in Table S1 **(**except for toluene), with a high relationship coefficient of R^2 = 0.90283.

As shown in **Table S1**, although the dielectric constant of toluene (2.4) is larger than that of dioxane (2.21), according to the Lippert-Mataga equation, the polarity parameter (Δf) of toluene (0.0153) is smaller than that of dioxane (0.0205), due to its higher refractive index (n = 1.496) than that of dioxane (n = 1.4224). In a lower polarity media, **CPM** would exhibit less charge separation and weaker interaction with solvents, leading to a stronger and shorter fluorescence emission.[1-3] In contrast, when the environment polarity increased, **CPM** would perform larger charge separation and the excited state energy can be dissipated due to the dipole-dipole interaction between the probe and solvent. As a result, the energy was rapidly consumed through nonradiative relaxation, leading to a weaker fluorescence and

longer emission wavelength. In our experiment, when the solvent polarity decreases from water (0.3212) to toluene (0.0153), the fluorescence quantum yield of **CPM** increases from 0.73 to 25.09 (Table S1 and Fig. S4 in revised SI). So the fluorescence quantum yield of **CPM** in toluene (25.09) is much higher than that of dioxane (10.53), making toluene is the outlier points.

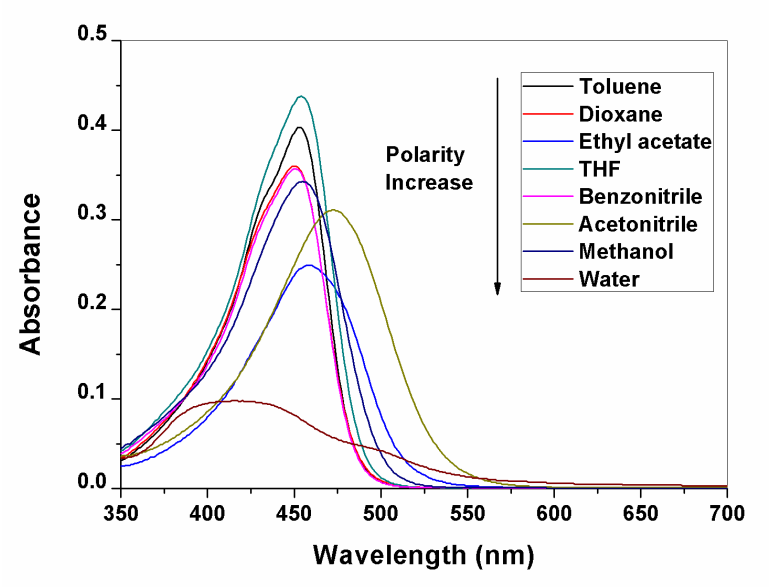


Fig. S3 Absorption spectra of **CPM** (10 μM) in different solvents.

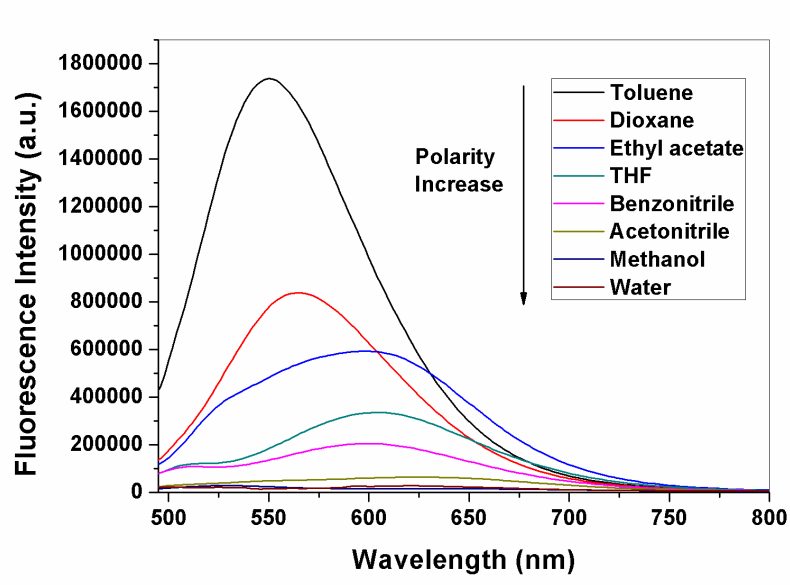


Fig. S4 Fluorescence emission spectra of **CPM** (10 μM) in different solvents (λ_{ex} = 465 nm).

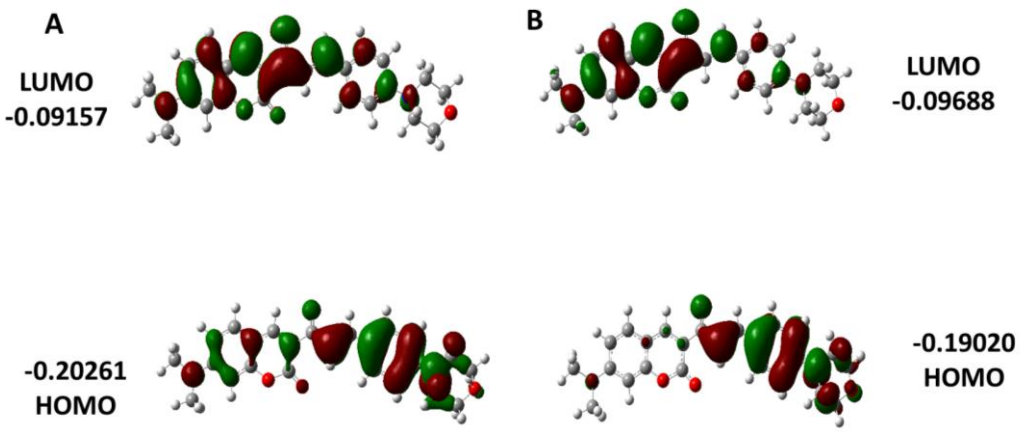


Fig. S5 HOMO and LUMO of **CPM** by DFT calculations at the base level of B3LYP/6-31G via Gaussian 09 program. (A) Ground State; (B) Excited State. The highest occupied molecular orbital (HOMO) of **CPM** is localized from the donor (D) morpholine part onto the phenylethylene linker (C=C), whereas the lowest unoccupied molecular orbital (LUMO) is localized on the acceptor coumarin group (A). **CPM** might undergo a substantial ICT process from morpholine to the coumarin group displaying a typical D-π-A structural feature, which is in accord with our design strategy.

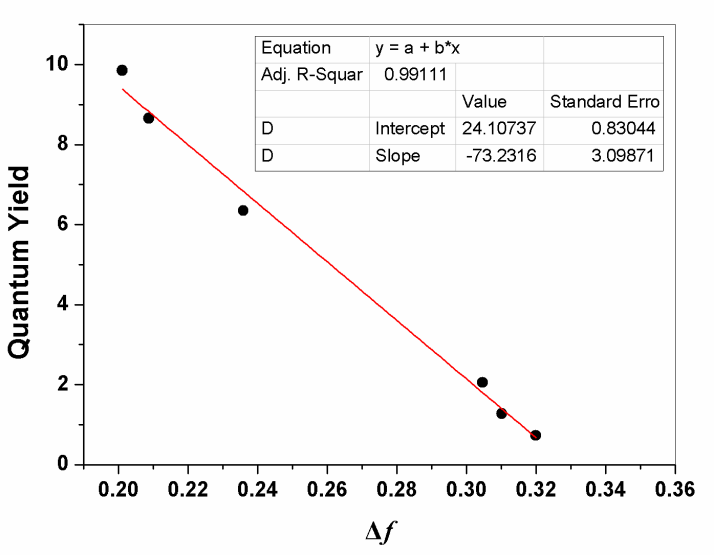


Fig. S6 Linearity of quantum yield of **CPM** (10 μM) versus the solvent parameter Δf in different solvents.

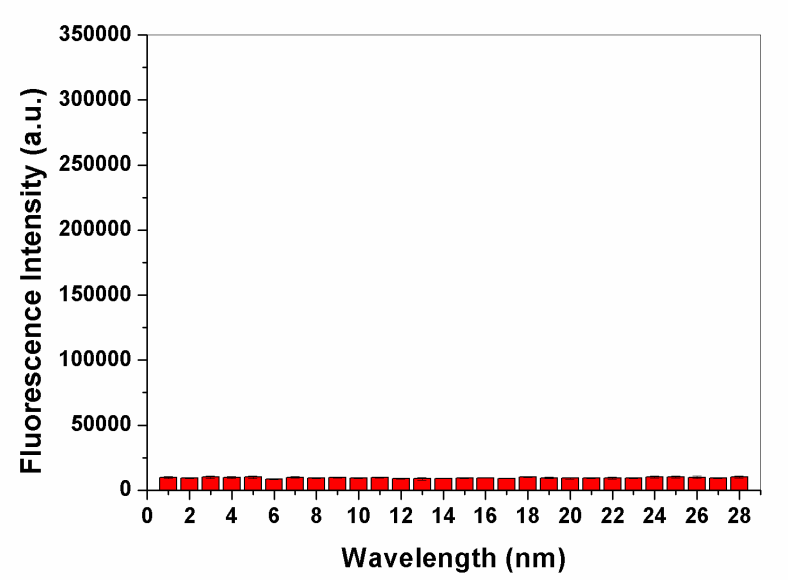


Fig. S7 The fluorescence intensity of **CPM** (10 μM) to various relevant analytes in phosphate buffer (pH 7.4). 1. Blank; 2, Ca^{2+}; 3, Na^{+}; 4, K^{+}; 5, Cu^{2+}; 6, Fe^{2+}; 7, Fe^{3+}; 8, Pb^{2+}; 9, Ba^{2+}; 10, Ag^{+}; 11, Cl^{-}; 12, NO_3^{-}; 13, SO_3^{2-}; 14, ClO^{-}; 15, ClO_4^{-}; 16, $ONOO^{-}$, 17, H_2O_2; 18, Arg^{-}; 19, Asn; 20, Asp; 21, Cys; 22, Thr; 23, Ser; 24, Gln; 25,Glu; 26, His; 27, Tyr; 28,GSH.

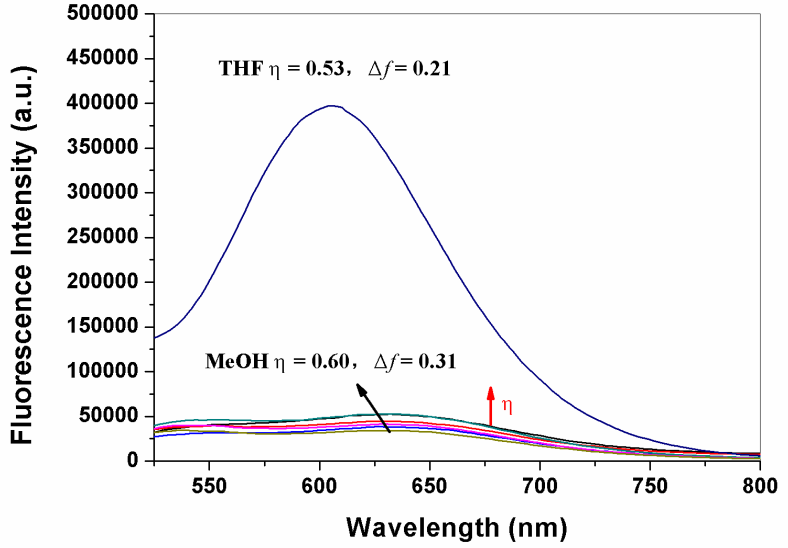


Fig. S8 The fluorescence spectra of **CPM** (10 μM) in methanol-glycerol system under different viscosity. THF and methanol have almost the same viscosity (0.53 cP vs 0.60 cP) but different polarity (0.21 vs 0.31). The fluorescence intensity of **CPM** displayed huge difference in them. The fluorescence intensity changed little with increasing viscosity from 0.60 cP to about 100 cP.

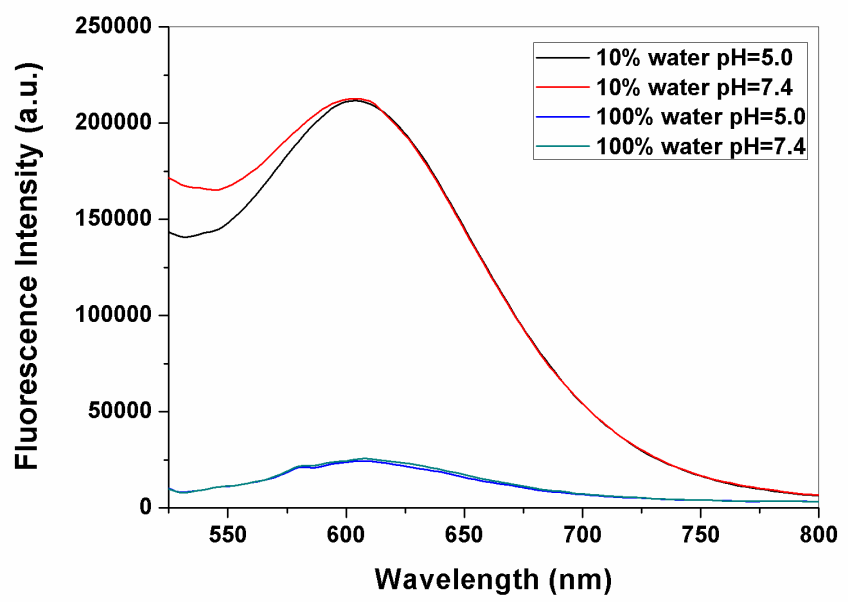


Fig. S9 The fluorescence spectra of **CPM** (10 μM) under different water contents (10% and 100%) in H_2O/THF solvent mixtures with different pH values (5.0 and 7.4). λ_{ex} = 465 nm.

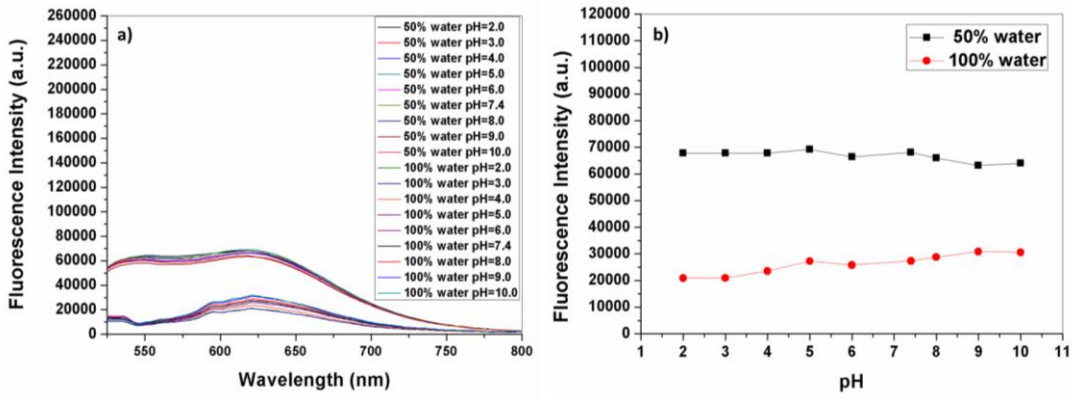


Fig. S10 a) The fluorescence spectra of **CPM** (10 μM) under different water contents (50% and 100%) in H_2O/THF solvent mixtures with different pH values (from pH 2.0 to pH 10.0). b) Changes in the fluorescence intensity of **CPM** under different water contents (50% and 100%) in H_2O/THF solvent mixtures with different pH values (from pH 2.0 to pH 10.0). λ_{ex} = 465 nm.

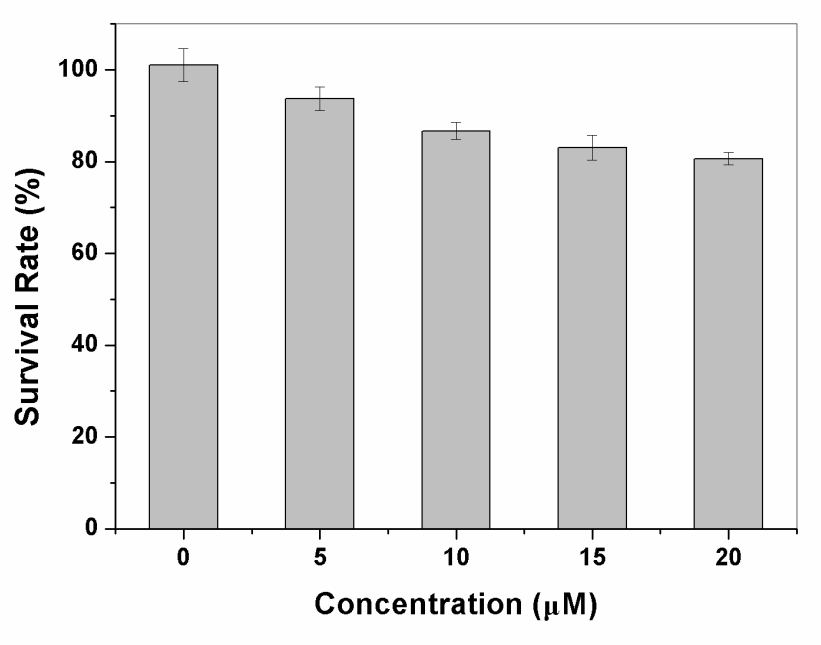


Fig. S11 Cell viability of **CPM** on SMMC-7721 cells by a standard *CCK-8* assay. 1, control; 2, 5 μM; 3, 10 μM; 4, 15 μM; 5, 20 μM. Data are expressed as mean values ± standard error of the mean of three independent experiments, each performed in three triplicate.

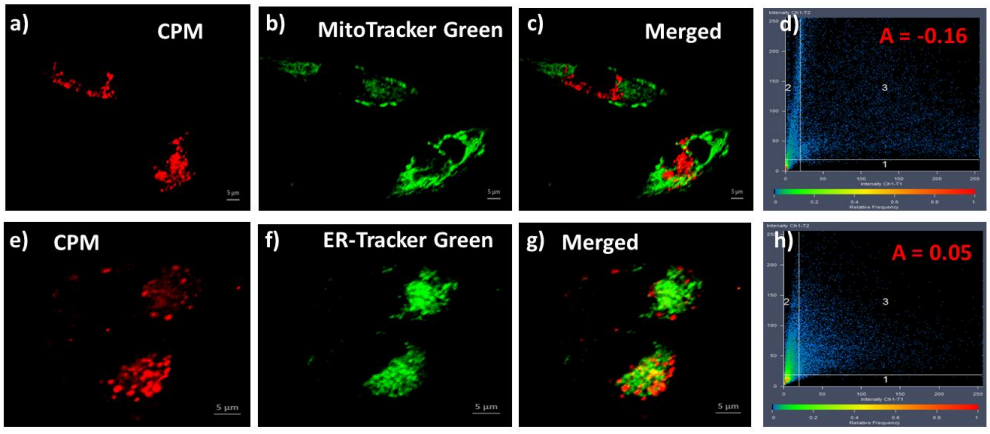


Fig. S12 Fluorescence images of 5 μM **CPM** (a, e), images of co-labeled with 0.2 μM MitoTracker Green (b), 1.0 μM ER-Tracker Green (f), merged images (c, g) in SMMC-7721 cells. The Pearson's co-localization correlations of **CPM** with MitoTracker Green (d, -0.16) and ER-Tracker Green (h, 0.05), respectively. The red channel image was collected at 560 - 650 nm (λ_{ex} = 458 nm) for **CPM**. The green channel image was collected at 496 - 536 nm (λ_{ex} = 488 nm) for MitoTracker Green, and ER-Tracker Green. Scale bar: 5 μm.

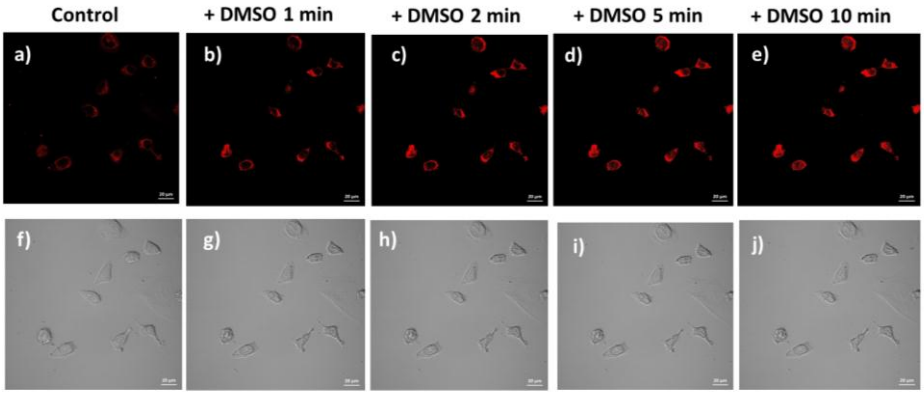


Fig. S13 Time-dependent fluorescence images of **CPM** in SMMC-7721 cells mediated by DMSO. Control cells with only **CPM** (3 μM) treated for 10 min (a), followed by treating with DMSO (10 μL) (b-e). The dark-field transmission images (f-j). The red channel images were collected at 560 - 650 nm (λ_{ex} = 458 nm). Scale bar: 20 μm.

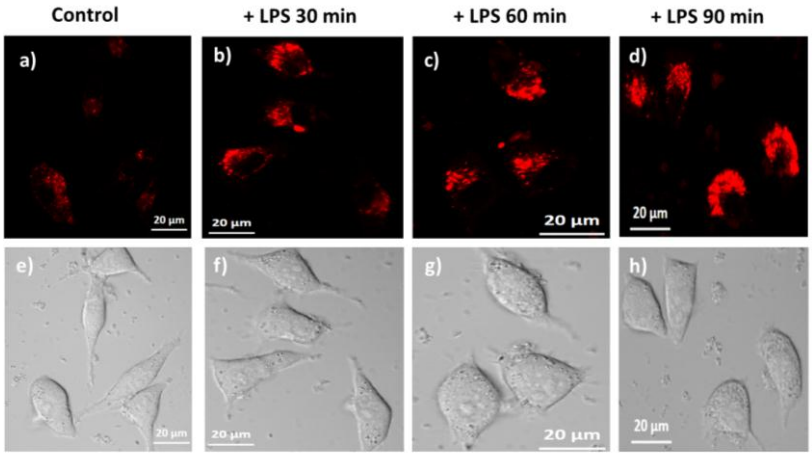


Fig. S14 Fluorescence images of **CPM** induced by LPS in live SMMC-7721 cells. (a) Control cells with only **CPM** (3 μM) incubated for 10 min. SMMC-7721 cells were mediated by LPS (50 μg/mL) for 30 min (b), 60 min (c), or 120 min (d), subsequently incubated with **CPM** (3 μM) for 10 min. (e-h) The bright-field cells images. The red channel images were collected at 560 - 650 nm (λ_{ex} = 458 nm). Scale bar: 20 μm.

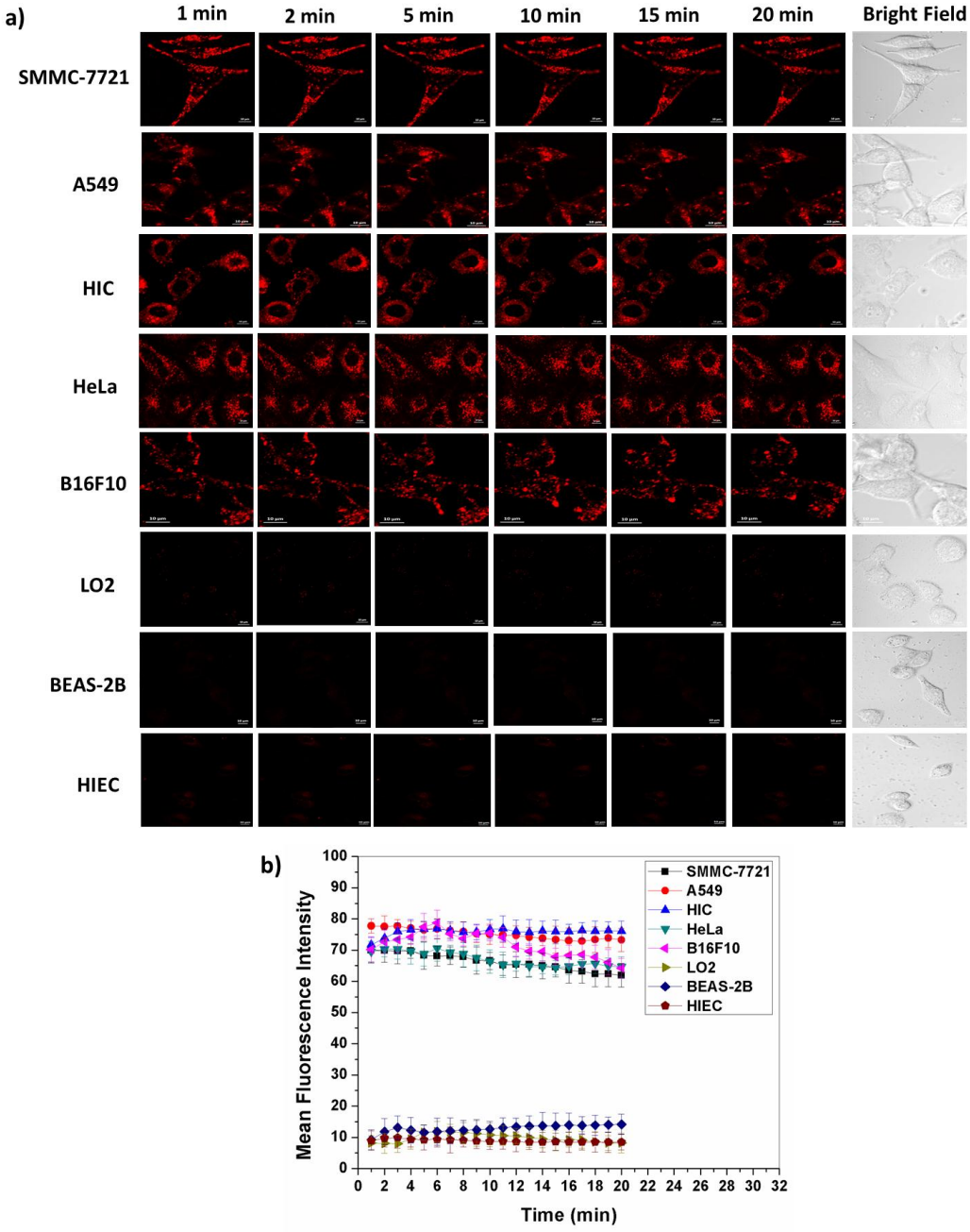


Fig. S15 (a) Fluorescence images of **CPM** in five types of cancer cells (SMMC-7721, A549, HIC, HeLa and B16F10 cells) and three types of normal cells (LO2, BEAS-2B and HIEC cells) during 20 min. The cells were incubated with 5 μM **CPM** for 10 min at 37 ℃ and then washed with RPMI1640 or DEME medium at pH 7.4. (b) Mean fluorescence intensities of **CPM**-stained live cells. Error bars represent mean deviation (± S. D.), n = 5. The red channel images were collected at 560 - 650 nm (λ_{ex} = 458 nm). Scale bar: 10 μm.

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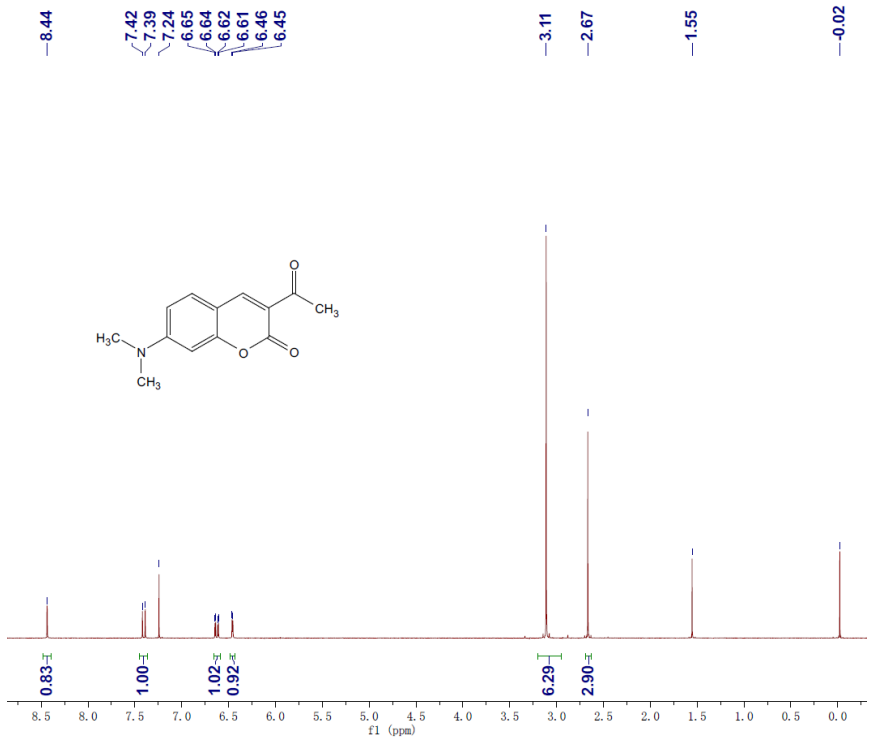
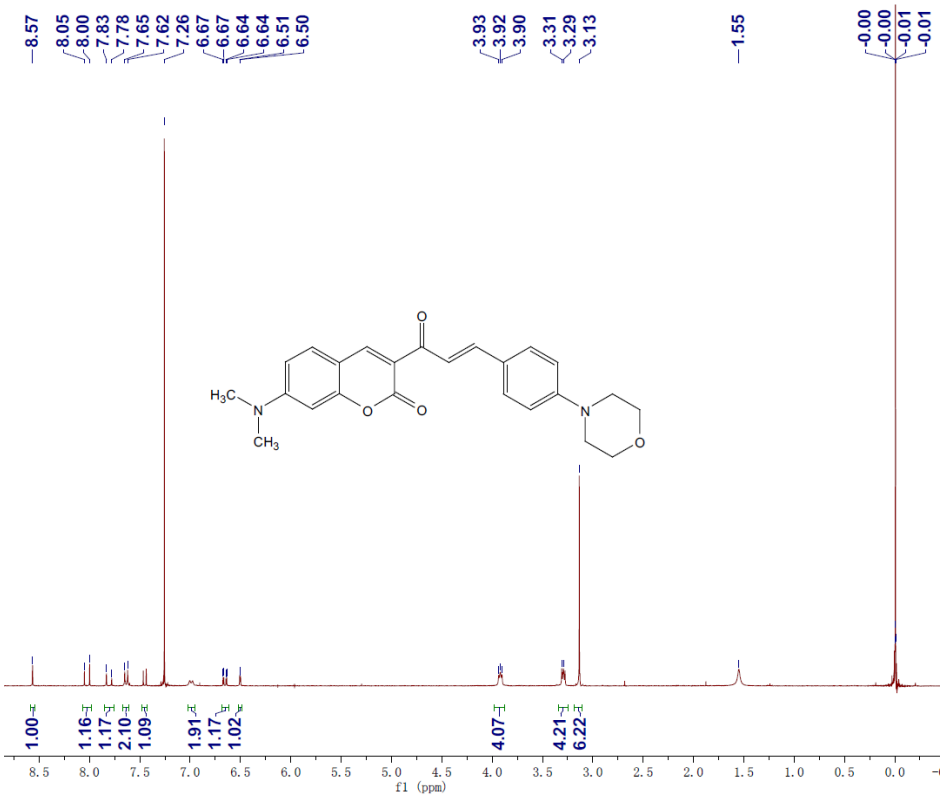
^{1}H NMR and ^{13}C NMR spectra and HR-MS analysis of compound 2-3 and CPM

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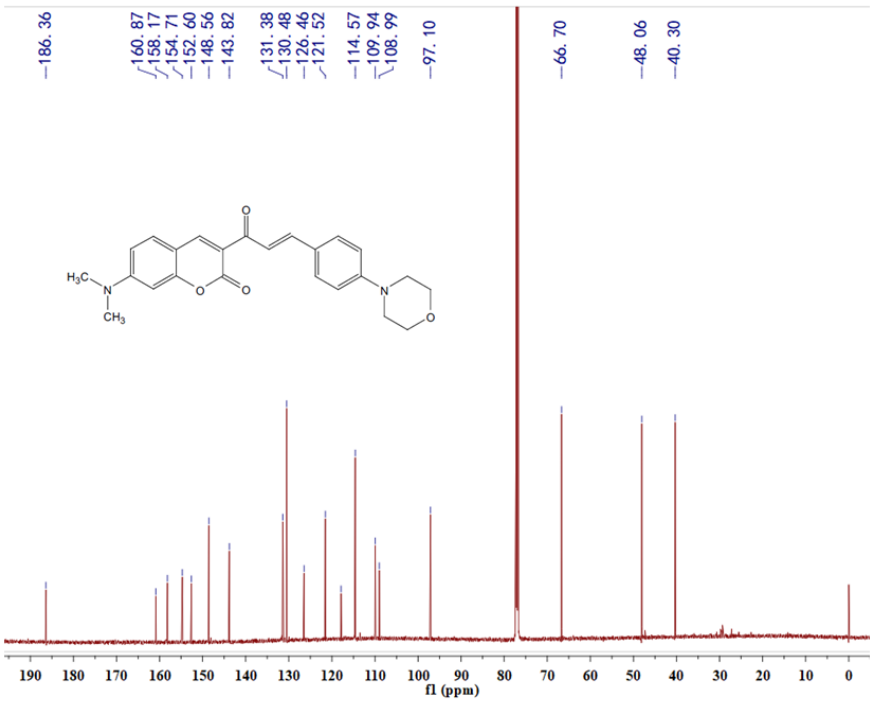
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126.46
121.52
114.57
109.94
108.99
97.10
66.70
48.06
40.30
f1 (ppm)

HIGH RESLUTION MASS SPECTROMETRY REPORT

Sample	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
CPM	$C_{24}H_{24}N_2O_4$	$[M+H]^+$	405.1805	405.1809	-0.99