Supporting Information

Fluorescent probe for lipid droplet polarity imaging with low viscosity crosstalk

Bo Lin[a], Zhenru Li[a], Qi Zan[b], Li Fan[b], Yang Shu[a]*, Jianhua Wang[a]*

[a] Department of Chemistry, College of Sciences, Northeastern University, Shenyang, 110819, China

[b] Institute of Environmental Science, Shanxi University, Taiyuan, 030006, China

E-mail address: shuyang@mail.neu.edu.cn (Shu), jianhuajrz@mail.neu.edu.cn (Wang).

Experimental

Materials and Chemicals

2,6-naphthalenediol and N,N'-diphenylformamidine were purchased from Bide Pharmaceutical Technology Co., Ltd. (Shanghai). Oleic acid (OA) and methyl-β-cyclodextrin (MβCD) were obtained from Meryer (Shanghai) Chemical Technology Co., Ltd. Piperidine, toluene, acetone, sulfuric acid, acetic acid, 1,4-dioxane, H_2O_2 (30%) and the silica gel of 200-400 mesh (the sorbent of column chromatography) were received from Sinopharm (Shenyang) Chemical Reagent Co., Ltd. 2-Benzothiazoleacetonitrile, lipopolysaccharide(LPS), the metal phosphate and potassium salt with various anions used in the selectivity evaluation were supplied by Aladdin (Shanghai) Reagent Co., Ltd. DNA and RNA used for the selectivity estimation were achieved from Sangon Biotech (Shanghai) Co., Ltd.. The culture mediums and fetal bovine serum (FBS) for cell culturing were supplied by Thermo Fisher Scientific (China) Co., Ltd.. Quinine was obtained from Macklin (Shanghai) Biochemical Technology Co., Ltd. Mito Tracker Deep Red were acquired from Invitrogen (China). Nystatin and erastin were provided by Med Chem Express (China). Glyceryl trioleate, nile red, amino acids and the reagents for cell experiments were commercially available from Sigma Aldrich (China Mainland). The solvents used were supplied via Shanghai Titan Scientific Co., Ltd. All the reagents/chemicals and solvents are at least of analytical-reagent or HPLC grade and used without further purification. The cell lines were purchased from National Collection of Authenticated Cell Cultures (China).

Instrumentation

Deionized water with resistivity of 18.2 $M\Omega \cdot cm^{-1}$ (25℃) was produced by Milli-Q water purification system (Millipore) and used throughout the experiments. ^1H NMR (400 MHz) was acquired on a Bruker NMR spectrometer (Bruker biospin, Switzerland). d^6-DMSO was used as solvent, tetramethylsilane (TMS) as an internal reference standard. 1260 Hip Degasser and 6540 UHD Accurate Mass QTOF tracking module (Agilent, USA) was employed for measuring mass spectra. UV-vis absorption spectra were recorded on a U-3900 double-beam UV-vis spectrophotometer (Hitachi High-

Techonologies Corporation, Japan). Fluorescence spectra were obtained using a RF-6000 spectrofluorometer (SHIMADZU Excellence in Science, Japan). A laser particle sizer (Malvern Nano-ZS90) was employed to implement the dynamic light scattering (DLS). Fluorescence imaging in live cells were implemented on a FV-1200 confocal laser scanning microscope (Olympus, Japan).

Preparation and characterization

BTHO and the intermediates were prepared according to the synthetic route in Scheme S1.

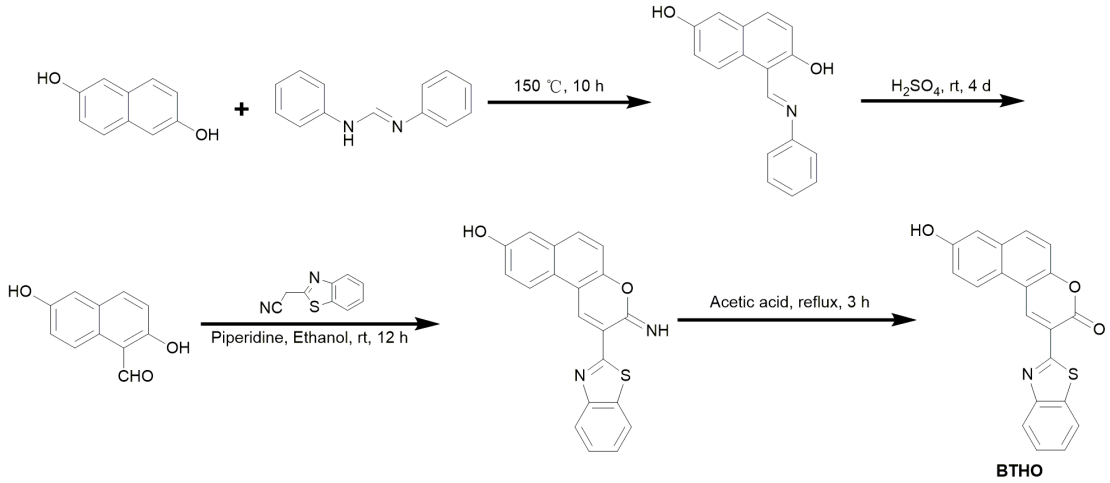
2,6-dihydroxy-1- naphthaldehyde:

This compound was synthesized according to the previously reported process[1]. The mixture of 2,6-naphthalenediol (10 mmol, 1.602 g) and N,N'-diphenylformamidine (12 mmol, 2.355 g) was heated to 150℃ and maintained for 10 h in a 35 mL thick-wall high-pressure bottle. The reaction mixture was dispersed in 30 mL acetone and sonicated for 30 min. Afterwards, filtered the dispersion system and collected the red-brown filter residue. The dried residue was mixed with 40 mL H_2O/H_2SO_4 at room temperature and stirred. After 4 days, 200 mL H_2O was added to quench the reaction. The mixture was extracted with dichloromethane (DCM) for 3 times and the organic layer was dried with anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was purified via silica gel column chromatography with n-hexane/ethyl acetate (10/1, v/v) and n-hexane/ethyl acetate (5/1, v/v) as eluent in order. The yellow solid product (1.051 g, 89%) was acquired. ^{1}H NMR (600 MHz, d^6-DMSO) δ 11.64 (s, 1H), 10.76 (s, 1H), 9.65 (s, 1H), 8.78 (d, J = 9.1 Hz, 1H), 7.94 (d, J = 9.1 Hz, 1H), 7.16 (ddd, J = 13.6, 6.8, 2.6 Hz, 3H). MS (Q-TOF) m/z: Calcd 188.1003, found 187.0925 $[M]^-$.

2-(benzothiazol-2-yl)-8-hydroxy-3H-benzochromen-3-one (BTHO):

BTHO was prepared by a similar procedure reported by Sarkar[2]. 2,6-Dihydroxy-1-naphthaldehyde (1 mmol, 0.1880 g), 2-benzothiazoleacetonitrile (1.3 mmol, 0.2265 g), 2.12 mL piperidine and 12.16 mL ethanol were mixed in a 50 mL round bottom flask. Afterwards, the mixture was stirred at room temperature for 12 h. The reaction mixture

was filtered and the solid washed 3 times by 30 mL n-hexane. Thereafter, the dried solid was dissolved in 35 mL acetic acid and further refluxed for 3h. After removing acetic acid, the residue was purified by silica gel column chromatography with n-hexane/ethyl acetate (6/1, v/v) as eluent. The yellow solid product was obtained after drying at vacuum (0.1932 g, 56%). ^{1}H NMR (600 MHz, DMSO) δ 10.12 (s, 1H), 9.76 (s, 1H), 8.56 (d, *J* = 9.1 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 8.15 (t, *J* = 8.2 Hz, 2H), 7.70 (ddd, *J* = 31.8, 5.7, 3.3 Hz, 1H), 7.65-7.57 (m, 2H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.39 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.35 (d, *J* = 2.2 Hz, 1H). MS (Q-TOF) m/z: Calcd 344.0939, found 345.1017 $[M]^-$.



Scheme S1. Schematic illustration for the preparation of BTHO.

Spectroscopic measurements

6.91 mg BTHO was dissolved in a small amount of DMSO (~ 10 mL) and the volume was adjusted to 20 mL for preparing 1.0 mM stock solution. Afterwards, the stock solution was stored at 4℃ for future use. The stock solution of BTHO was diluted to 10 μM with various solvents for measuring the absorption and fluorescence spectra. The resulting solutions were shaken for 12 h and then kept still for 2 h. 3 mL of the above solutions were placed in a quartz optical cell with a 1 cm optical path length, after which the absorption and fluorescence spectra were recorded. For measuring the fluorescence of BTHO, the excitation and emission bandwidths were both set at 5.0 nm. All spectroscopic experiments were carried out at room temperature. The excitation and emission wavelengths were the corresponding optimal values in the assessing the effect

of solvation on BTHO's fluorescence. In other fluorescence measurement, the excitation wavelength was all fixed at 405 nm to match the cell imaging.

The samples for evaluating the effect of polarity on BTHO's absorption and emission spectra were obtained by mixing DMSO and 1,4-dioxanein different volume proportions. In addition, these absorption and emission spectra of BTHO were also measured in the mixed systems of DMSO and toluene, water and 1,4-dioxane, and water and DMSO, respectively, for confirming the established relationship between the fluorescence and polarity of BTHO.

The solutions of interfering substances for selectivity test were freshly prepared by using the solution containing 10 μM BTHO. PB/DMSO (1:1, v/v) with 0.01 M phosphate and pH 7.40 were selected as solvent for acquiring sufficient fluorescence intensities of the probe and solubility of interfering substances. The cations and anions were derived from their phosphate and potassium salts, respectively. Among them, the concentrations of Na^+ and K^+ were 10 mM and 140 mM respectively, and the concentrations of other ions were 1 mM. The concentrations of all DNA were 100 μM. The concentrations of RNA-1 and RNA-2 were 10 and 50 μM, respectively. The concentrations of proteins were fixed at 1 mg/L. The fluorescence intensities at 493 nm were recorded at stabilization.

The H_2O/DMSO (1/1, v/v), DMSO, ethyl acetate (EA), toluene and 1,4-dioxane solutions of 10 μM BTHO were selected for assessing its photostability. The time-dependent fluorescence intensity at maximum emission wavelength was simultaneously collected with 1.08 s intervals during 3 h under continuous light irradiation. The power of the Xenon Lamp is 150 W.

Calculation of fluorescence quantum yield

The relative quantum yield of BTHO was calculated according to the following equation:

$$\Phi_{\mathrm{x}} = \Phi_{\mathrm{st}} \times \frac{\mathrm{D_x}}{\mathrm{D_{st}}} \times \frac{A_{st}}{A_x} \times \frac{n_{Dx}^2}{n_{Dst}^2} \quad \text{(S1)}$$

Where Φ_{st}, D, A and n_{D} represent the reported quantum yield of the standard, the

integrated fluorescence intensity of the emission spectra, the absorbance at the excitation wavelength and the refractive index of the solvent used, respectively. Subscripts *x* and *st* stand for the sample and the standard, respectively. Quinine sulfate solution (Φ = 0.54 in 0.1 M H_2SO_4) is used as reference standard.

Measurement of hydration diameter

The stock solution of the probe was diluted to 10 μM by water and shocking 2 h. Then, the mixture was rested again for 2 h and used for detection of dynamic light scattering (DLS). The hydrated particle sizes were recorded on a laser particle sizer.

Cytotoxicity assay of BTHO

BTHO cytotoxicity to NCM 460 cells (Human normal intestinal epithelial cells) was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide) assay. NCM460 cells were transplanted in 96-well flat-bottomed plates and cultured in 1640 medium containing 10% (v/v) FBS at the atmosphere of 5% CO_2 and 37℃. The total volume in each well was controlled at 200 μL, and a cell density of 1×10^5 cells per well was maintained. After 24 h, the medium was removed and these cells were washed with PBS for 3 times. 1 μL of BTHO (DMSO) with different concentrations (0, 0.005, 0.02, 0.2, 1, 2, 3, 4, 5, 6 mM) were mixed with 199 μL 1640 medium. Then, these culture mediums were perfused to each well and incubated with the cells for 24 h. 10 μL of MTT solutions (10 mg/mL) were added into each well and further incubated for 4 h. Subsequently, these culture mediums were removed and 150 μL of DMSO was added into each well to dissolve the blue-violet formazan crystals produced with the cells. The absorbance at 490 nm was recorded on a microplate reader.

Dynamic assays of cells stained by BTHO

NCM460 cells were seeded in glass Petri dish and cultured in 1640 medium containing 10% FBS at 37℃ in 5% CO_2 atmosphere for 24 h. Afterwards, the medium was removed and the cells were washed for 3 times via PBS. Then, 5 μL stock solution of BTHO (1.0 mM) was added into the glass petri dish containing 1.0 mL Hanks medium (Hanks balanced salt solution, HBSS) with a final concentration of 5 μM. Subsequently, the time-dependent fluorescence images of BTHO in the cyan channel

was collected at $\lambda_{ex}/\lambda_{em}$ = 405/(440-540) nm. The imaging was conducted with the frequency of times/(1 min) during 20 min on a FV-1200 confocal laser scanning microscope with 100× objective lens.

Photostability evaluation of the probe in live cells

NCM460 cells were transplanted in glass Petri dish and incubated in 1640 medium supplemented with 10% FBS at 37℃ in 5% CO_2 atmosphere for 24 h. Then, the redundant culture medium was cleaned and the cells were rinsed for 3 times by PBS. Subsequently, 5 μL of the stock solution of BTHO was poured into the petri dish containing 1.0 mL Hanks medium with a final concentration of 5 μM. After 30 min, the continuous fluorescence imaging was implemented. The images in the cyan channel with 440-540 nm were collected 100 times with 30 s intervals during 50 min under continuous laser radiation of 405 nm on a FV-1200 confocal laser scanning microscope with 100× objective lens. Fixed 405 laser power: 50×25% = 12.5 mW.

The physical parameters of solvents and photo-physical properties of BTHO in these solvents

Table S1. The physical parameters of solvents at 20℃.

Solvent	$\boldsymbol{\varepsilon}$[a]	$\mathbf{n_D^2}$[b]	$\boldsymbol{\Delta f}$[c]	$\boldsymbol{\eta}$[d] (mPa·s)
Water	80.20	1.7769	0.4054	1.00
DMSO	46.60	2.1845	0.3738	2.47
Glycerol	46.50	2.1697	0.3745	1499
DMF	38.25	2.0449	0.3795	0.90
Ethanol	24.55	1.8523	0.3744	1.17
Acetone	20.70	1.8469	0.3199	0.32
DCM	9.08	2.0306	0.2924	0.43
EA	6.02	1.8824	0.1424	0.45
Toluene	2.39	2.2410	0.1222	0.59
1,4-Dioxane	2.21	2.0164	0.4054	1.3

[a] The dielectric constant of solvents. [b] n_D is the refractive indices of solvents. [c] $\Delta f = \frac{\varepsilon-1}{2\varepsilon+1} - \frac{n_D^2-1}{4n_D^2+2}$ is the orientational polarizability of solvents. [d] The viscosity of solvents.

Table S2. Photo-physical properties of BTHO in various solvents at 20°C.

Solvent	**λ_{abs}[e] (nm)**	**ε_{max}[f] ($M^{-1}cm^{-1}$)**	**λ_{em}[g] (nm)**	**Stokes Shift (nm)**	**Φ_f[h]**	**$\varepsilon \times \Phi_f$[i] ($M^{-1}cm^{-1}$)**
DMSO	425	33660	515	90	0.01	673
Glycerol	423	34650	512	89	0.17	5891
DMF	423	34650	511	88	0.04	1386
Ethanol	422	35310	505	83	0.03	1059
Acetone	420	36630	500	80	0.37	13553
DCM	420	39270	502	82	0.67	26311
EA	418	39600	492	74	0.64	25344
Toluene	421	40260	493	72	0.80	32208
1,4-Dioxane	418	40590	488	70	0.94	38155
Glyceryl Trioleate	421	40260	493	72	-	-

[e] The maximal absorption wavelength of the probe. [f] The extinction coefficient of theprobe at the maximum absorption. [g] The maximal emission of the probe. [h] Φ_f is the relative fluorescence quantum yield derived with quinine sulfate (Φ_f = 0.54 in 0.1 M H_2SO_4) as a fluorescence standard [3]. [i] $\varepsilon \times \Phi_f$ represent for molecular brightness[4].

Table S3. The physical parameters of DMSO/1,4-Dioxane mixed solvents at 20℃.

%DMSO (Vol)	**%1,4-Dioxane (Vol)**	ε_{mix}[j]	n_{Dmix}^2[k]	Δf_{mix}[l]
100	0	46.60	1.7769	0.3738
95	5	44.38	1.7889	0.3734
90	10	42.16	1.8008	0.3730
85	15	39.94	1.8128	0.3725
80	20	37.72	1.8248	0.3718
75	25	35.50	1.8368	0.3711
70	30	33.28	1.8487	0.3702
65	35	31.06	1.8607	0.3691
60	40	28.84	1.8727	0.3677
55	45	26.62	1.8847	0.3661
50	50	24.40	1.8966	0.3641
45	55	22.18	1.9086	0.3616
40	60	19.97	1.9206	0.3585
35	65	17.75	1.9326	0.3545
30	70	15.53	1.9445	0.3493
25	75	13.31	1.9565	0.3423
20	80	11.09	1.9685	0.3323
15	85	8.87	1.9805	0.3175
10	90	6.65	1.9924	0.2931
5	95	4.43	2.0044	0.2464
0	100	2.21	2.0164	0.1222

[j] The dielectric constant of mixed solvents. [k] n_{Dmix} stands for the refractive indices of mixed solvents. [l] The orientational polarizability of mixed solvents.

Table S4. The physical parameters of DMSO/Toluene mixed solvents at 20 ℃.

%DMSO (Vol)	**%Toluene (Vol)**	ε_{mix}	n_{Dmix}^2	Δf_{mix}
100	0	46.60	2.1845	0.3738
95	5	44.40	2.1873	0.3728
90	10	42.20	2.1901	0.3718
85	15	40.00	2.1930	0.3707
80	20	37.79	2.1958	0.3695
75	25	35.59	2.1986	0.3682
70	30	33.39	2.2014	0.3667
65	35	31.19	2.2043	0.3650
60	40	28.99	2.2071	0.3631
55	45	26.79	2.2099	0.3609
50	50	24.58	2.2127	0.3583
45	55	22.38	2.2156	0.3553
40	60	20.18	2.2184	0.3517
35	65	17.98	2.2212	0.3472
30	70	15.78	2.2241	0.3416
25	75	13.58	2.2269	0.3342
20	80	11.37	2.2297	0.3242
15	85	9.17	2.2325	0.3097
10	90	6.97	2.2354	0.2867
5	95	4.77	2.2382	0.2446
0	100	2.57	2.2410	0.1424

Table S5. The physical parameters of H_2O/1,4-Dioxane mixed solvents at 20 ℃.

%H_2O (Vol)	**%1,4-Dioxane (Vol)**	ε_{mix}	n_{Dmix}^2	Δf_{mix}
100	0	80.20	1.7769	0.4054
95	5	76.30	1.7889	0.4041
90	10	72.40	1.8008	0.4027
85	15	68.50	1.8128	0.4013
80	20	64.60	1.8248	0.3998
75	25	60.70	1.8368	0.3982
70	30	56.80	1.8487	0.3966
65	35	52.90	1.8607	0.3948
60	40	49.00	1.8727	0.3929
55	45	45.10	1.8847	0.3908
50	50	41.20	1.8966	0.3885
45	55	37.30	1.9086	0.3859
40	60	33.41	1.9206	0.3828
35	65	29.51	1.9326	0.3792
30	70	25.61	1.9445	0.3747
25	75	21.71	1.9565	0.3689
20	80	17.81	1.9685	0.3609
15	85	13.91	1.9805	0.3491
10	90	10.01	1.9924	0.3291
5	95	6.11	2.0044	0.2862
0	100	2.21	2.0164	0.1222

Table S6. The physical parameters of H_2O/DMSO mixed solvents at 20 ℃.

%H_2O (Vol)	**%DMSO (Vol)**	ε_{mix}	n_{Dmix}^2	Δf_{mix}
100	0	80.20	1.7769	0.4054
90	10	76.84	1.8176	0.4021
80	20	73.48	1.8584	0.3989
70	30	70.12	1.8992	0.3957
60	40	66.76	1.9399	0.3925
50	50	63.40	1.9807	0.3894
40	60	60.04	2.0214	0.3863
30	70	56.68	2.0622	0.3832
20	80	53.32	2.1030	0.3801
10	90	49.96	2.1437	0.3770
0	100	46.60	2.1845	0.3738

Molecule coordinates of BTHO from output results of the Gaussian 09 package and Lippert-Metaga equation

Table S7. Molecule coordinates of BTHO in DMSO, ground state.

Number	Atomic Number	X	Y	Z
1	6	4.22757400	0.36981200	0.03775500
2	6	2.94836900	-0.27379200	0.05678100
3	6	1.75835000	0.54272800	-0.00941800
4	6	1.89873200	1.92812000	-0.08877700
5	6	3.15800100	2.56402000	-0.10446400
6	6	4.29177600	1.79434000	-0.04300200
7	6	5.41034600	-0.40260800	0.09717300
8	6	5.34555700	-1.78143500	0.17404500
9	6	4.08554500	-2.42459900	0.19482400
10	6	2.92132900	-1.68663300	0.13727300
11	8	6.51547800	-2.48028300	0.22752500
12	6	0.42415900	0.03745500	0.01112100
13	6	-0.67966400	0.84904100	-0.05074000
14	6	-0.50556900	2.30198200	-0.15503800
15	8	0.81186000	2.74630700	-0.15560600
16	8	-1.36350400	3.15194000	-0.25465600
17	6	-2.03819800	0.29687300	-0.03198000
18	7	-3.09205400	0.90180800	0.42648100
19	6	-4.22164000	0.10938300	0.32520700
20	6	-4.02040800	-1.17253600	-0.24535200
21	16	-2.33387400	-1.35158700	-0.67713600
22	12	-5.50764800	0.48645700	0.74508300
23	12	-6.55693000	-0.41076100	0.58778500
24	12	-6.34299200	-1.67959700	0.01829300
25	12	-5.07527600	-2.07513800	-0.40342300
26	1	3.20082700	3.64591800	-0.16625200
27	1	5.26808800	2.27049400	-0.05614200
28	1	6.37855000	0.08860800	0.08121000
29	1	4.03653000	-3.50912400	0.25520000
30	1	1.97616400	-2.21823800	0.15389400
31	1	6.32568200	-3.43176900	0.26955000
32	1	0.27168000	-1.03303500	0.09920200
33	1	-5.65997700	1.46757000	1.18404200
34	1	-7.55607400	-0.13053000	0.90803900
35	1	-7.17833200	-2.36428900	-0.09549700
36	1	-4.91594900	-3.05525900	-0.84141500

Table S8. Molecule coordinates of BTHO in DMSO and excited state.

Number	Atomic Number	X	Y	Z
1	6	4.22403700	0.39160300	-0.00028400
2	6	2.94674500	-0.26197700	0.00007600
3	6	1.73968600	0.53050700	0.00000300
4	6	1.89934700	1.95335200	-0.00032000
5	6	3.14454600	2.57856600	-0.00063000
6	6	4.30166200	1.81271800	-0.00064400
7	6	5.39693600	-0.38818800	-0.00025900
8	6	5.33427600	-1.78948400	0.00015900
9	6	4.08501700	-2.43833400	0.00059000
10	6	2.92672900	-1.68023700	0.00053500
11	8	6.50989700	-2.45214400	0.00019400
12	6	0.43379500	0.02111200	0.00020000
13	6	-0.71137400	0.86556100	0.00027300
14	6	-0.51831800	2.31084600	0.00031300
15	8	0.81112500	2.77071000	-0.00034500
16	8	-1.35152400	3.19248000	0.00078700
17	6	-2.03316000	0.33261600	0.00023900
18	7	-3.17794400	0.99712800	0.00048900
19	6	-4.25801300	0.16602300	0.00024000
20	6	-3.97772900	-1.23697300	-0.00025500
21	16	-2.24897900	-1.47913700	-0.00040900
22	12	-5.60561800	0.59070800	0.00039000
23	12	-6.61570700	-0.35901000	0.00006000
24	12	-6.31909200	-1.73848100	-0.00041700
25	12	-4.99794800	-2.18732100	-0.00057900
26	1	3.18347900	3.66282900	-0.00085400
27	1	5.27552400	2.29219900	-0.00091400
28	1	6.37112400	0.09074400	-0.00055000
29	1	4.03153300	-3.52307900	0.00099600
30	1	1.97867400	-2.20621000	0.00089600
31	1	6.35613000	-3.41283500	0.00045300
32	1	0.28450700	-1.05063100	0.00051700
33	1	-5.82572900	1.65376800	0.00076200
34	1	-7.65363900	-0.03853300	0.00016500
35	1	-7.12866700	-2.46232100	-0.00065200
36	1	-4.77454500	-3.24964500	-0.00093700

Table S9. Molecule coordinates of BTHO in 1,4-dioxane and ground state.

Number	Atomic Number	X	Y	Z
1	6	4.14286800	0.35466900	0.10691200
2	6	2.87691800	-0.29016500	0.10077700
3	6	1.69922800	0.51846400	0.08102700
4	6	1.81624700	1.91889800	0.05123000
5	6	3.06613600	2.52979200	0.06900100
6	6	4.21990200	1.75336100	0.09408600
7	6	5.32593800	-0.40304400	0.12808900
8	6	5.28886600	-1.79514700	0.14052800
9	6	4.06561600	-2.44843600	0.13049800
10	6	2.87981500	-1.70839700	0.11019200
11	8	6.44144600	-2.50726200	0.16230800
12	6	0.34318200	-0.04162100	0.12242200
13	6	-0.69420400	0.77664900	-0.07720500
14	6	-0.52130400	2.23966700	-0.32237800
15	8	0.75103900	2.78045300	0.04508700
16	8	-1.39749800	2.92096600	-0.79921900
17	6	-1.93039400	0.26774400	-0.05673400
18	7	-2.77582500	0.54615200	0.90860700
19	6	-3.97298700	-0.11284900	0.67601300
20	6	-4.01320100	-0.89295600	-0.48302000
21	16	-2.49275000	-0.78032000	-1.28746700
22	12	-5.11923000	-0.05786700	1.49496400
23	12	-6.26514200	-0.77670400	1.13876100
24	12	-6.28217800	-1.54677900	-0.01990600
25	12	-5.15897700	-1.61876700	-0.85001900
26	1	3.14190200	3.62716400	0.06321200
27	1	5.20462800	2.24348100	0.10397100
28	1	6.29786400	0.11204200	0.13495900
29	1	4.02847000	-3.54777900	0.13851200
30	1	1.91850200	-2.24299600	0.10123400
31	1	6.21103600	-3.45154100	0.16804500
32	1	0.18411300	-1.11309000	0.31386700
33	1	-5.11248000	0.54914100	2.41229400
34	1	-7.15862700	-0.73286400	1.77888700
35	1	-7.19095400	-2.10628500	-0.28651100
36	1	-5.17216700	-2.22755100	-1.76610100

Table S10. Molecule coordinates of BTHO in 1,4-dioxane and excited state.

Number	Atomic Number	X	Y	Z
1	6	4.25086700	0.38299600	-0.01826700
2	6	2.97376600	-0.27281200	0.11204300
3	6	1.76277300	0.49631100	0.07960800
4	6	1.91498100	1.91505000	-0.09461700
5	6	3.15458900	2.53936700	-0.25287400
6	6	4.32205200	1.79465800	-0.20504600
7	6	5.41481900	-0.40062500	0.03142700
8	6	5.35106500	-1.80103400	0.19370900
9	6	4.10538500	-2.45541200	0.30765300
10	6	2.95758300	-1.69402900	0.26536400
11	8	6.53233300	-2.44154200	0.22480100
12	6	0.45468800	-0.01618800	0.18690500
13	6	-0.67487500	0.82417000	0.06909300
14	6	-0.51131900	2.23940600	-0.11503200
15	8	0.82578900	2.72615700	-0.07703300
16	8	-1.33912000	3.08940400	-0.37442400
17	6	-2.04237800	0.29206000	0.04611400
18	7	-3.06636300	0.78091300	0.69154800
19	6	-4.21196300	0.05274200	0.46244700
20	6	-4.06957000	-1.08480900	-0.38292300
21	16	-2.40444100	-1.18818000	-0.92351900
22	12	-5.47115900	0.34333200	1.01877800
23	12	-6.54599400	-0.48402400	0.72613900
24	12	-6.39044500	-1.60570400	-0.11223600
25	12	-5.15205200	-1.91648200	-0.67170600
26	1	3.16903400	3.61454600	-0.38762700
27	1	5.28988200	2.27193600	-0.30409900
28	1	6.39331100	0.05765800	-0.05666700
29	1	4.05667800	-3.53319700	0.42188600
30	1	2.00734700	-2.20591800	0.34559600
31	1	6.40268200	-3.39942800	0.33625600
32	1	0.28815000	-1.07245300	0.34878400
33	1	-5.57522600	1.21112200	1.66006100
34	1	-7.52237300	-0.26564300	1.14672500
35	1	-7.24567200	-2.23843100	-0.32619900
36	1	-5.03710800	-2.78170100	-1.31547000

Lippert-Metaga equation[5]:

$$hc\tilde{\nu}_{max} = -\frac{2\mu_e(\mu_e-\mu_g)}{\alpha^3}\Delta f + C \quad \text{(S2)}$$

Where h, c, $\tilde{\nu}_{\max}$, μ_e and μ_g are Planck constant, the light speed in vacuum, the solvent-equilibrated fluorescence maxima (wave number), the dipole moments of excited state and the dipole moments of ground state, respectively. Δf is the orientational polarizability of solvents described above and is positively correlated to the dielectric constant ε. α and C represent the Onsager cavity radius and a constant, separately. Here, the mean solute polarizabilities in the excited and ground states were neglected.

The FRET efficiency from BTHO to nile red in living cells

Table S11. Fluorescence intensities of BTHO before and after added nile red (**Fig. 4**), as well as the FRET efficiency in living cells.

Number	F_D **(0 min)**	F_{DA} **(18 min)**	E_{FRET}	**Average** E_{FRET}
1	48.59	0.09	0.97	
2	58.53	3.14	0.95	0.96 ± 0.01
3	51.89	0.133	0.97	

The FRET efficiency (E_{FRET}) was quantified by following equation[6,7]:

$$E_{FRET} = \frac{\alpha R_0^6}{\alpha R_0^6 + R^6} = 1 - \frac{F_{DA}}{F_D} \quad \text{(S3)}$$

E_{FRET} is obtained by measuring fluorescence intensities (F) of the donor in the presence (F_{DA}) and absence (F_D) of the acceptor; R (Å) stands for the separation distance between the donor and acceptor, Förster distance (R_0, Å) is the separation distance (R) of a single FRET pair corresponding to 50% energy transfer efficiency. α represents for the number of acceptor.

.

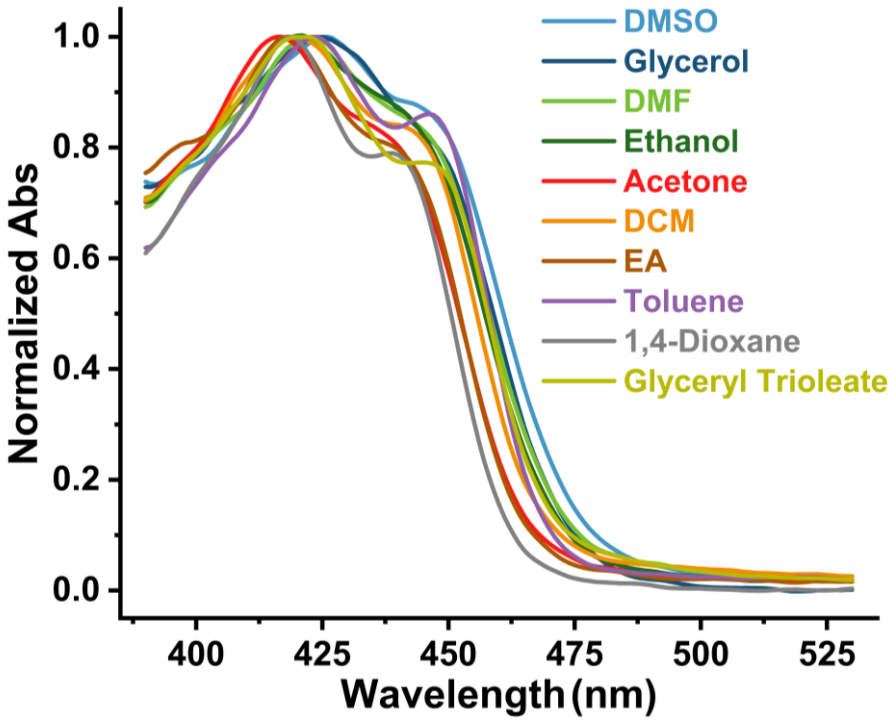


Fig. S1 UV-vis absorption spectra of BTHO (10 μM) in various solvents.

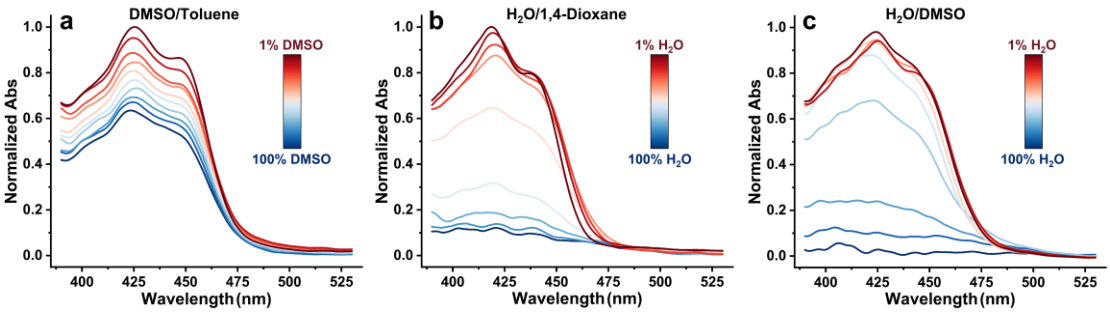


Fig. S2 UV-vis absorption spectra of BTHO (10 μM) in various mixture systems: (a) DMSO/Toluene; (b) H_2O/1,4-Dioxane; (c) H_2O/DMSO.

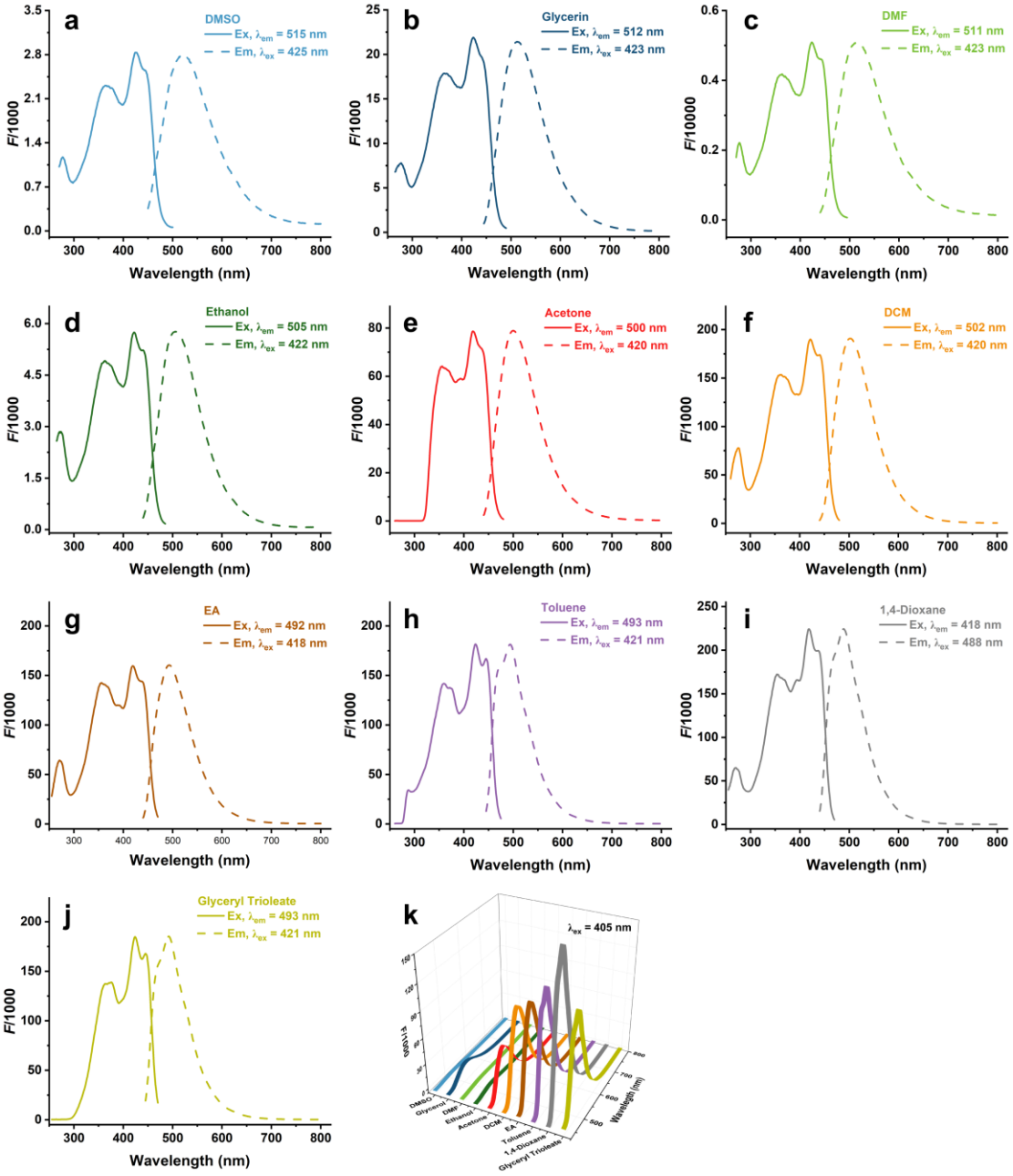


Fig. S3 Excitation and emission spectra of BTHO (10 μM) in various solvents. (a), DMSO; (b), Glycerin; (c), DMF; (d), Ethanol; (e), Acetone; (f), DCM, (CH_2Cl_2); (g), EA (ethyl acetate); (h), Toluene; (i), 1,4-Dioxane; (j), Glyceryl Trioleate. (k) The emission spectra of BTHO (10 μM) in the above solvents upon λ_{ex} = 405 nm.

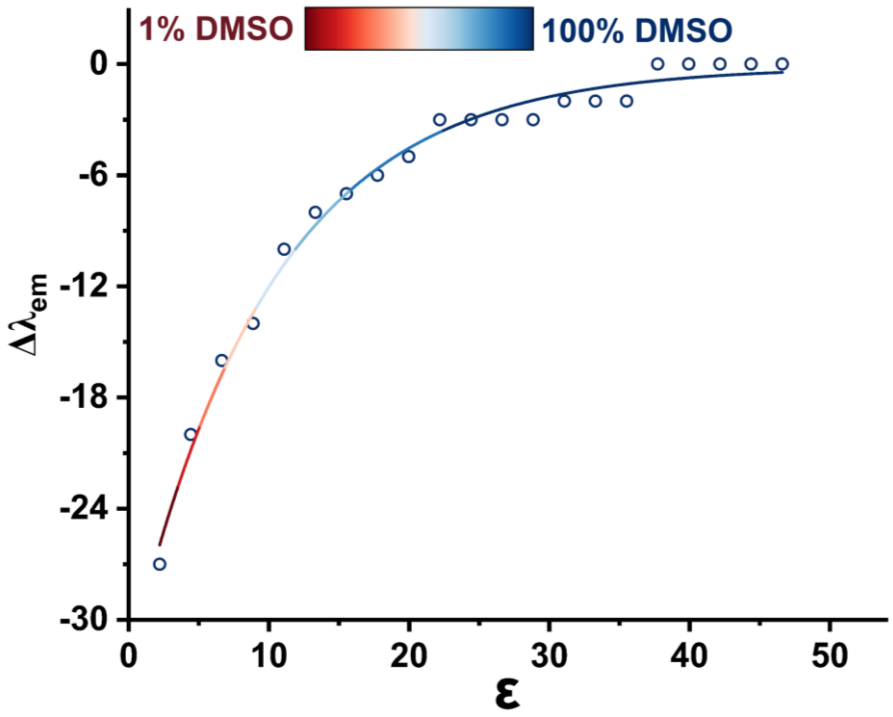


Fig. S4 Boltzmann function fitting curve of $\Delta\lambda_{em}$ vs. the dielectric constant. $\Delta\lambda_{em} = \lambda_{em}\ (in\ an\ organic\ solvent) - \lambda_{em}\ (in\ DMSO)$.

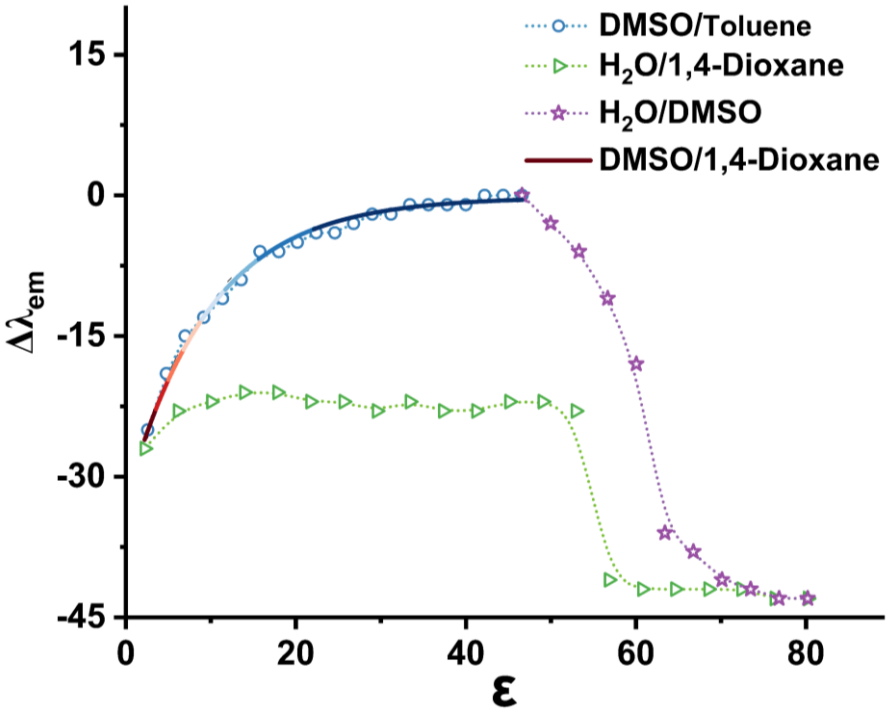


Fig. S5 The relationships between $\Delta\lambda_{em}$ with the dielectric constants of solvents in various mixture systems. $\Delta\lambda_{em} = \lambda_{em}\ (in\ an\ organic\ solvent) - \lambda_{em}\ (in\ DMSO)$.

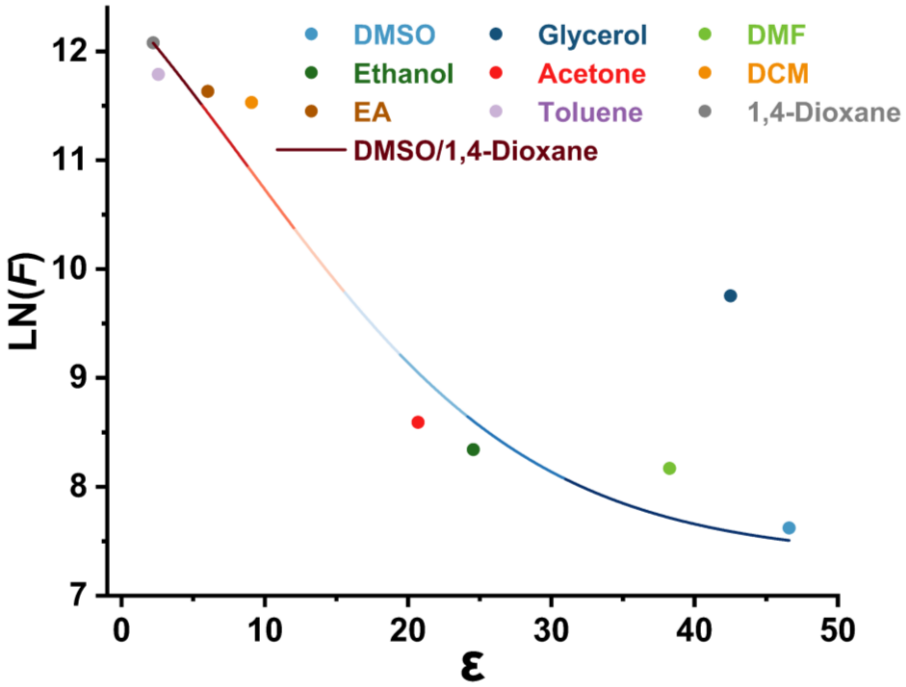


Fig. S6 The relationships between LN(*F*) and the dielectric constants of solvents. *F* stands for the fluorescence intensities of 10 μM BTHO at 493 nm. λ_{ex} = 405 nm.

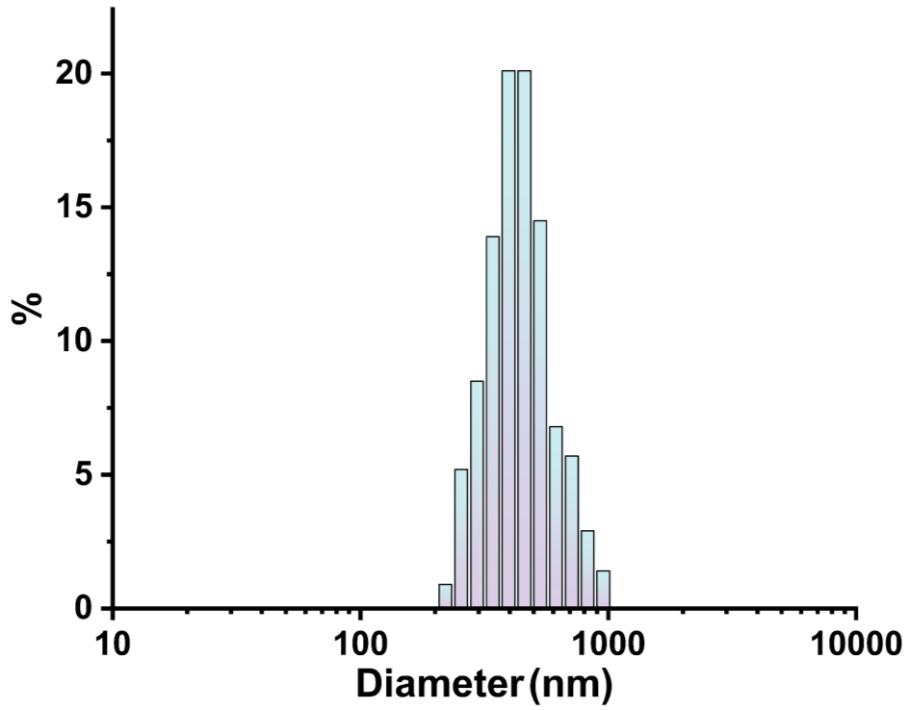


Fig. S7 The distribution of the hydrated particle size for 10 μM BTHO in aqueous medium.

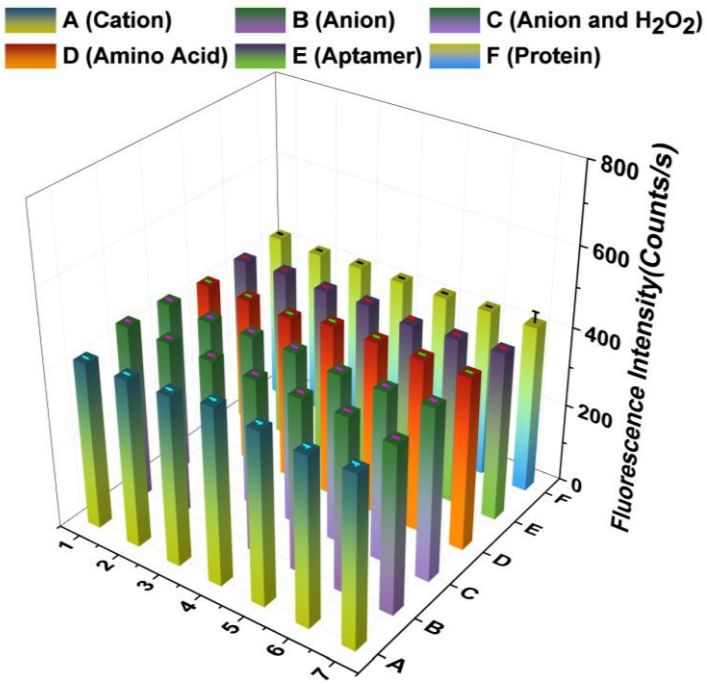


Fig. S8 The selectivity assessment for 10 μM BTHO in PB/DMSO (1:1, v/v) with 0.01 M phosphate and pH 7.40 against various common interfering substances. Excitation and emission were set at 405 and 493 nm, respectively. A1, Na^+(10 mM); A2, K^+ (140 mM); A3, Mg^{2+} (1 mM); A4, Ba^{2+} (1 mM); A5, Li^+ (1 mM); A6, Ni^{2+} (1 mM); A7, Er^{3+} (1 mM); B1, F^- (1 mM); B2, Cl^- (1 mM); B3, Br^- (1 mM); B4, I^- (1 mM); B5, SO_3^{2-} (1 mM); B6, SO_4^{2-} (1 mM); B7, HS^- (1 mM); C1, NO_2^- (1 mM); C2, NO_3^- (1 mM); C3, HCO_3^- (1 mM); C4, Ac^- (1 mM); C5, ClO_4^- (1 mM); C6, ClO^- (1 mM); C7, H_2O_2 (1 mM); D1, Glu (1 mM); D2, GSH (1 mM); D3, Cys (1 mM); D4, Tyr (1 mM); D5, Lys (1 mM); D6, His (1 mM); D7, Ala (1 mM); E1, DNA 1 (100 μM); E2, DNA 2 (100 μM); E3, DNA 3 (100 μM); E4, DNA 4 (100 μM); E5, DNA 5 (100 μM); E6, RNA 1 (10 μM); E7, RNA 2 (50 μM); F1, NTR (1 mg/L); F2, LDH (1 mg/L); F3, TB (1 mg/L); F4, Try (1 mg/L); F5, BSA (1 mg/L); F6, Hb (1 mg/L); F7, Cyt C (1 mg/L).
DNA 1 (bglf):
TAATACGACTCACTATAAGTCGCAGGAGTCGGGGGCGCAGAT;
DNA 2 (anti-bglf):
ATGTTATCTGCGCCCCCGACTCCTGCGACTTATAGTGAGTCGTA;
DNA 3 (anti-B30):
TGCACCACCACCACCACCACCACCACCACCACCAGTCGCA;
DNA 4 (anti-B12):
AGTCGCAGGAGTCGGGGGCGCAGATAACATTTTTTTTCCC;
DNA 5 (anti-B30G):
ACCACCACCACCACCACCACCACCACCACCTGTCGCAGGA;
RNA 1 (miR10b):
UACCCUGUAGAACCGAAUUUGUG;
RNA 2 (miR21):
UAGCUUAUCAGACUGAUGUUGA.

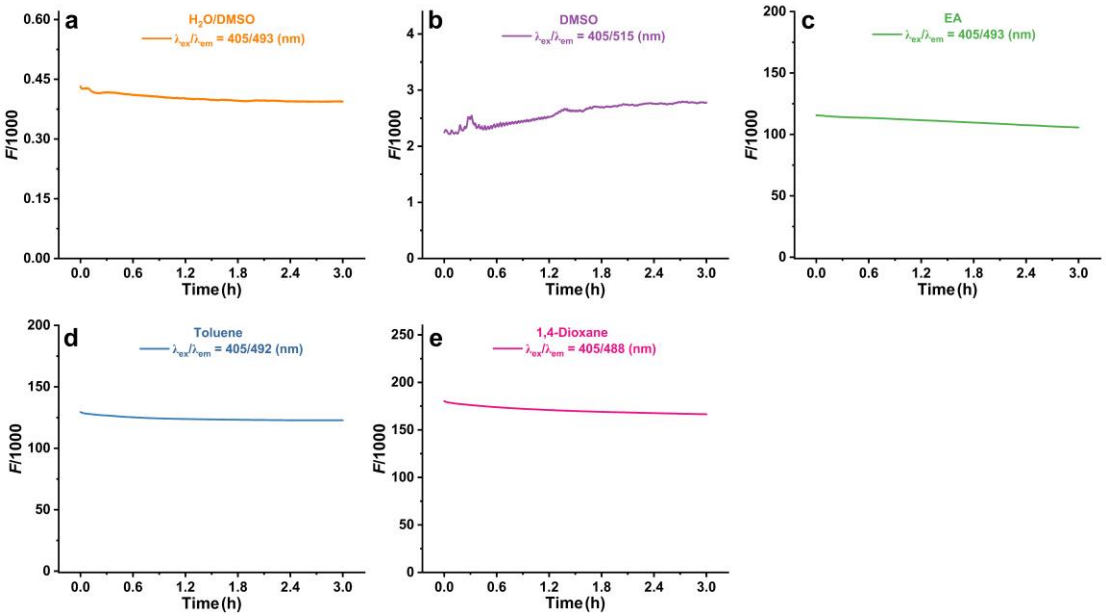


Fig. S9 The photostability appraisal for 10 μM BTHO in H_2O/DMSO (1:1, v/v, **a**), DMSO (**b**), EA(**c**), toluene (**d**) and 1,4-dioxane (**e**).

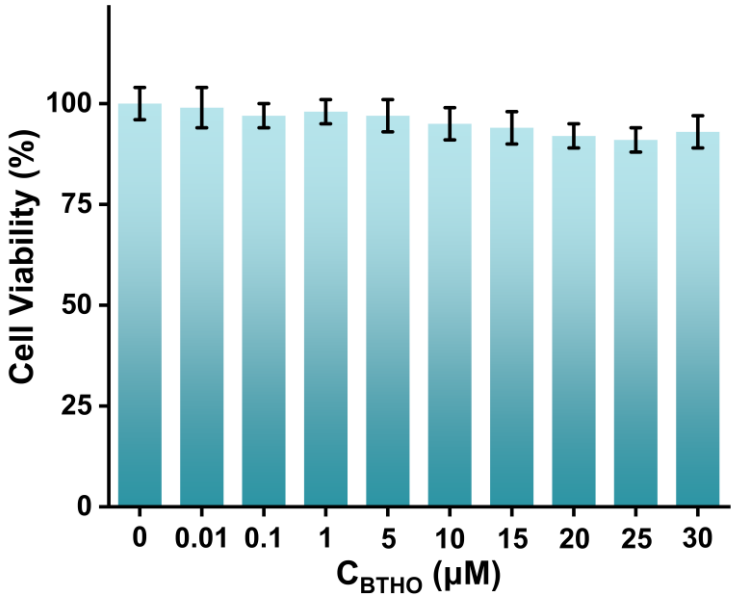


Fig. S10 MTT assay results of live NCM460 cells in the presence of BTHO at various concentrations.

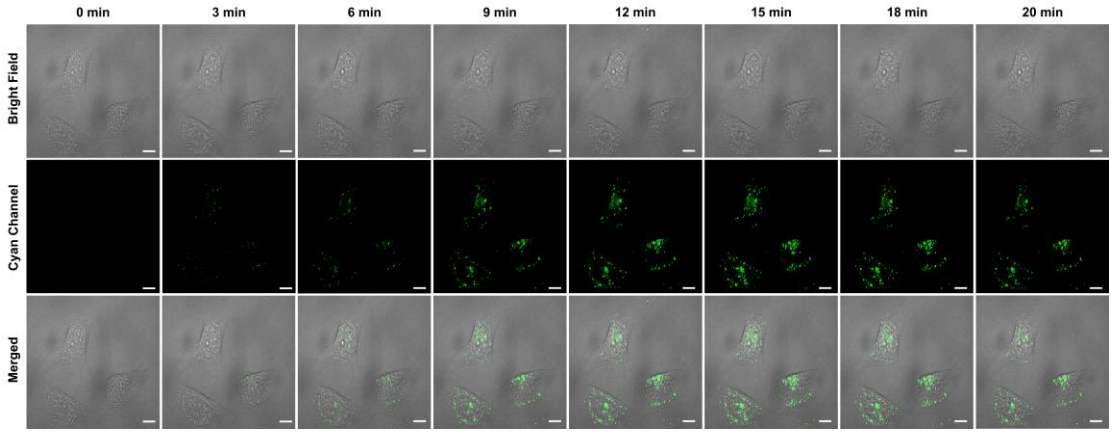


Fig. S11 The time-dependent fluorescence images of NCM460 cells after introducing 5 μM BTHO. Fluorescence emissions at the cyan channel (pseudo color) were collected at $\lambda_{ex}/\lambda_{em}$=405/(440-540) nm. Scale bar: 10 μm.

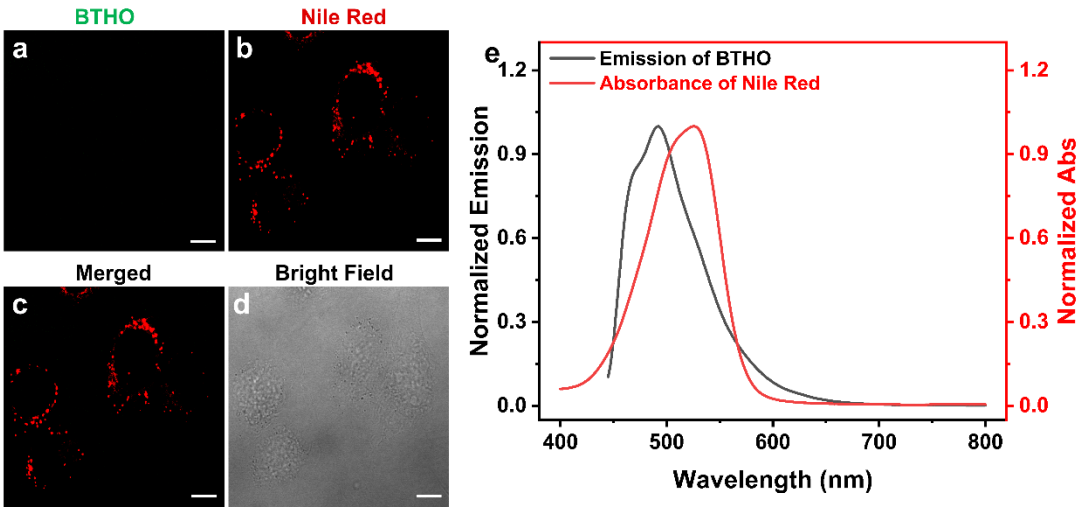


Fig. 12 (a-d) The confocal imaging of NCM460 cells. (a) After staining by 5 μM BTHO, (b) co-staining by 5 μM nile red, (c) the merged image, (d) the bright field image. The fluorescence images were obtained on an Olympus FV1200 confocal laser scanning microscope with the cyan channel ($\lambda_{ex}/\lambda_{em}$=405/(410-485 nm)) for BTHO, and the red channel ($\lambda_{ex}/\lambda_{em}$=488/(625-725 nm)) for nile red. Scale bars: 10 μm. (e) The fluorescence emission spectrum of BTHO (10 μM) in glyceryl trioleate under excitation with λ_{ex}=425 nm and the absorption spectrum of nile red (10 μM) in glyceryl trioleate.

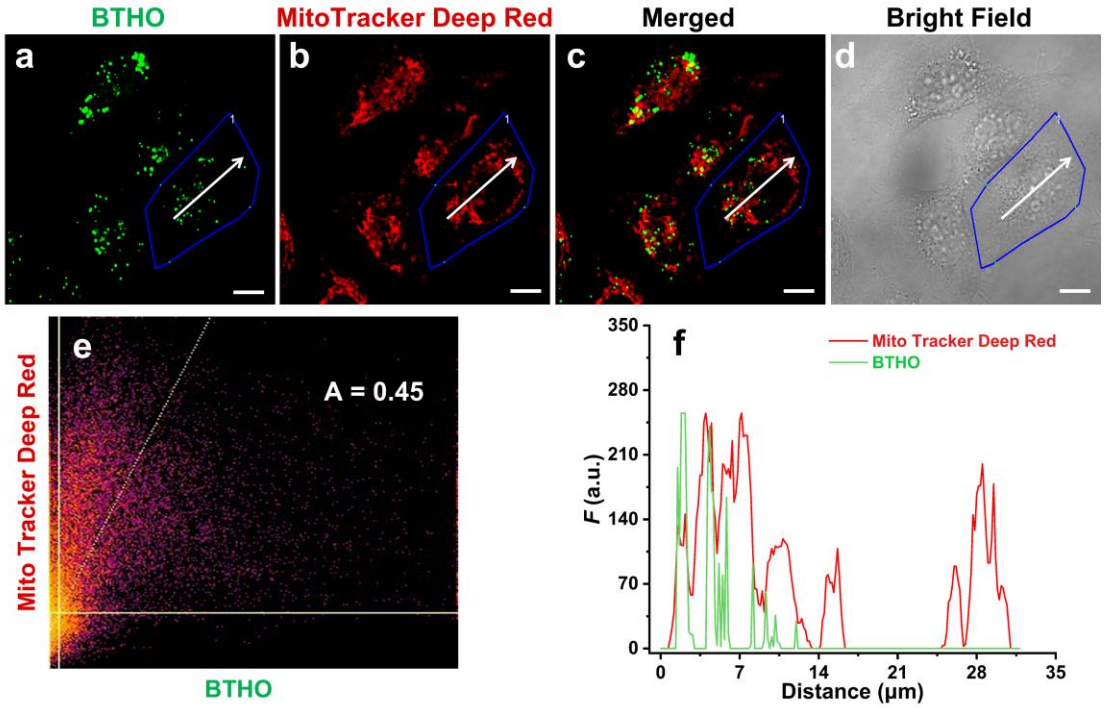


Fig. S13 Confocal fluorescence images of NCM460 cells. (a) After staining by 5 μM BTHO, (b) staining by 2 μM Mito Tracker Deep Red, (c) the merged image, (d) the bright field image. (e) The correlation of intensity distribution of BTHO and Mito Tracker Deep Red (Pearson Correlation Coefficient, A=0.45) within the blue ROI. (f) Intensity profile of the linear regions of interest across the NCM460 cells (the white arrow, sectional view). The fluorescence images were obtained on an Olympus FV1200 confocal laser scanning microscope with the cyan channel at $\lambda_{ex}/\lambda_{em}$=405/(440-540) nm for BTHO, and the deep red channel at $\lambda_{ex}/\lambda_{em}$=635/(640-725) nm for Mito Tracker Deep Red. Scale bars: 10 μm.

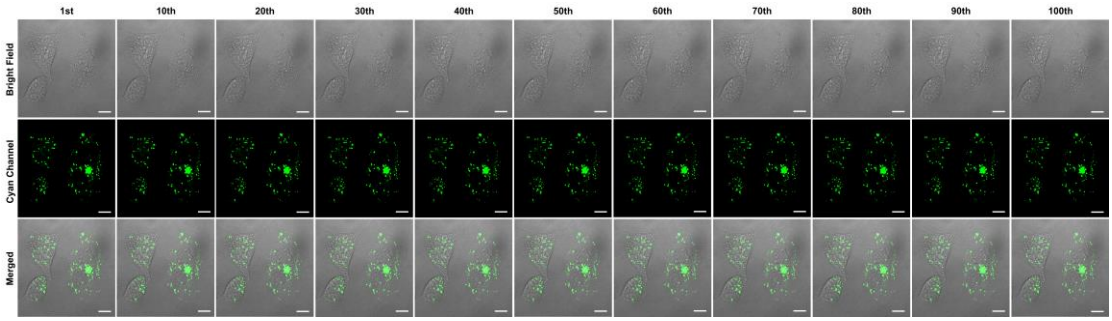


Fig. S14 The time-dependent fluorescence images of NCM460 cells pre-treated with 5 μM BTHO. Fluorescence emissions at the cyan channel (pseudo color) were collected at $\lambda_{ex}/\lambda_{em}$=405/(440-540) nm. Scale bar: 10 μm. Fixed 405 laser power: 50 × 25% = 12.5 mW.

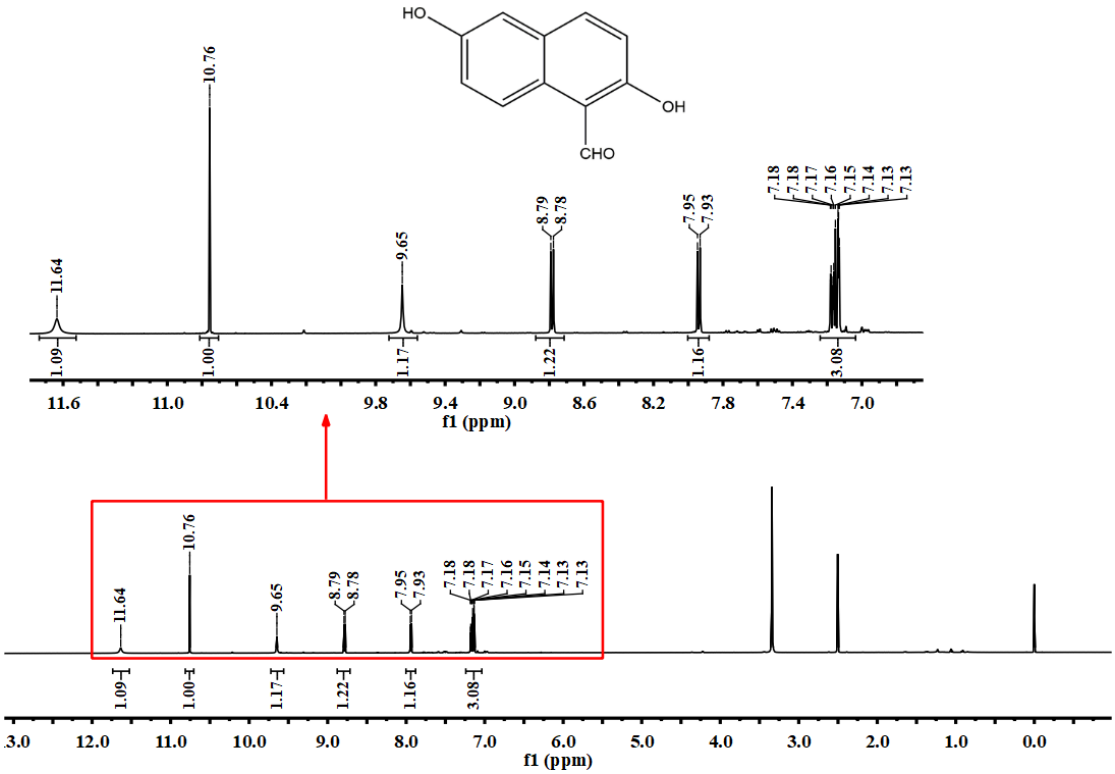


Fig. S15 ^{1}H NMR spectra of 2,6-dihydroxy-1-naphthaldehyde in d^{6}-DMSO.

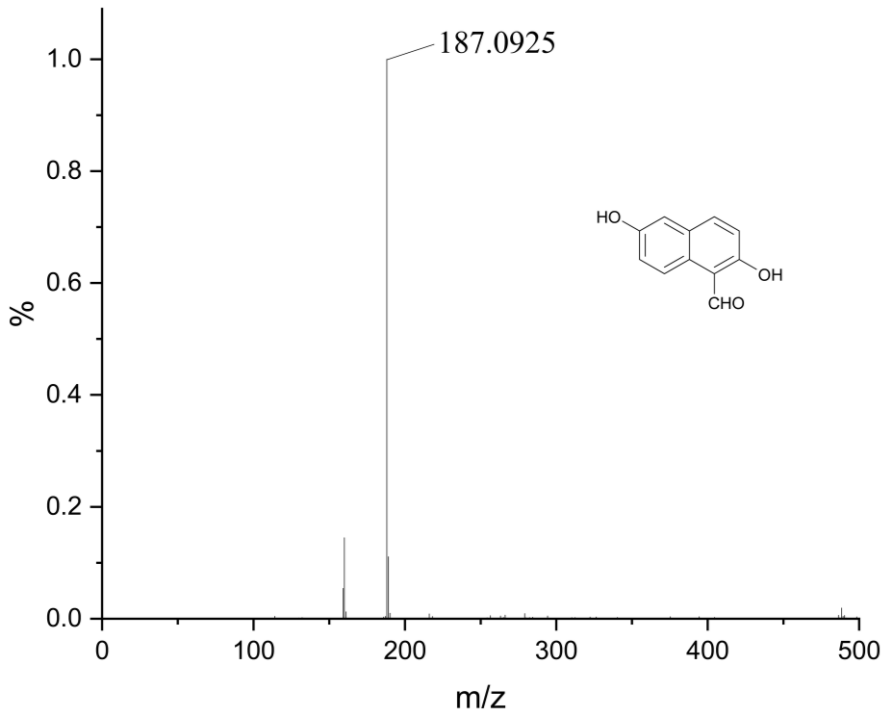


Fig S16 The mass spectra of 2,6-dihydroxy-1-naphthaldehyde ($[M]^-$).

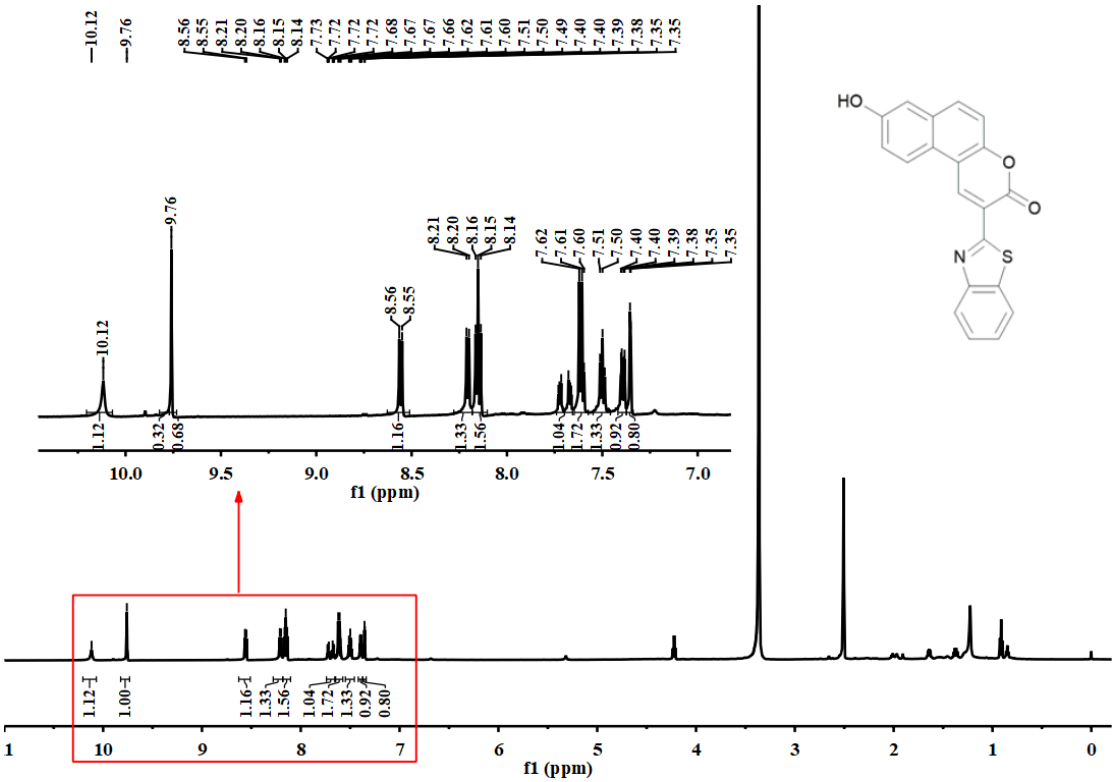


Fig. S17 ^{1}H NMR spectra of BTHO in d^{6}-DMSO.

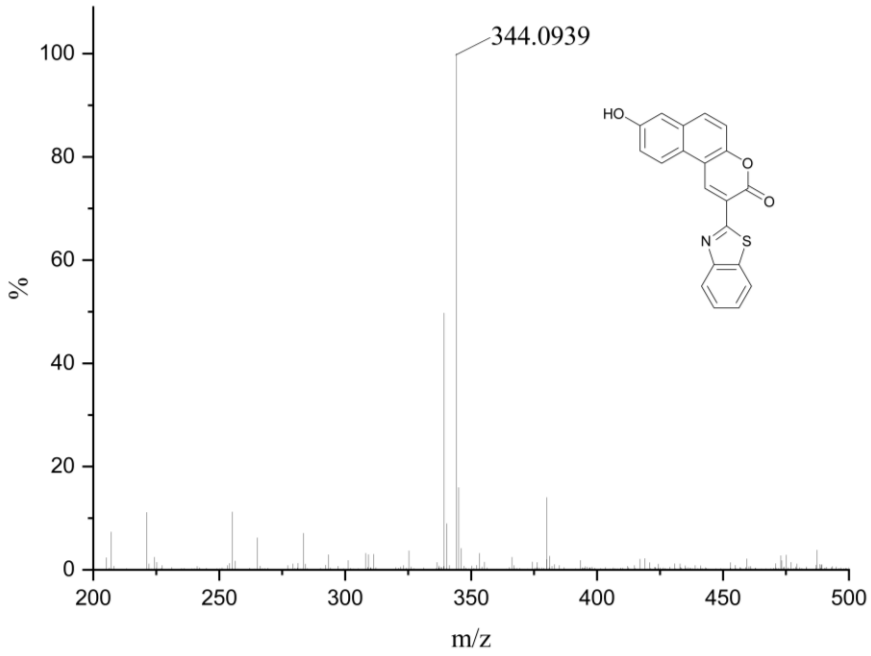


Fig. S18 The mass spectra of BTHO ($[M]^-$).

Reference

1. C. Dax, F. Duffieux, N. Chabot, M. Coincon, J. Sygusch, P. A. M. Michels and C. Blonski, *Journal of Medicinal Chemistry*, 2006, 49, 1499-1502.
2. S. Sarkar, M. Santra, S. Singha, Y. W. Jun, Y. J. Reo, H. R. Kim and K. H. Ahn, *Journal of Materials Chemistry B*, 2018, 6, 4446-4452.
3. W. H. Melhuish, *The Journal of Physical Chemistry*, 1961, 65, 229-235.
4. J. B. Grimm and L. D. Lavis, *Nature Methods*, 2022, 19, 149-158.
5. M. K. Singh, H. Pal, A. C. Bhasikuttan and A. V. Sapre, *Photochemistry and Photobiology*, 1998, 68, 32-38.
6. B. Fang, Y. Shen, B. Peng, H. Bai, L. Wang, J. Zhang, W. Hu, L. Fu, W. Zhang, L. Li and W. Huang, *Angewandte Chemie International Edition*, 2022, 61, e202207188.
7. L. Yuan, W. Lin, K. Zheng and S. Zhu, *Accounts of Chemical Research*, 2013, 46, 1462-1473.