Electronic Supplementary Information

A lipophilic AIEgen for lipid droplet imaging and evaluation of efficacy of HIF-1 targeting drugs†

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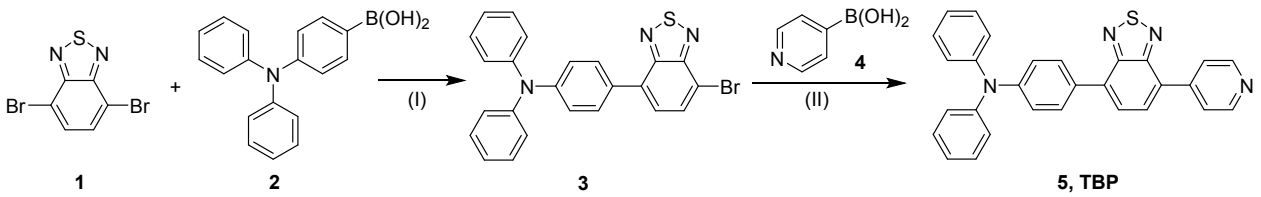

Scheme S1. The synthetic steps of TBP. (I and II) K_2CO_3, $Pd(PPh_3)_4$ and THF/H_2O.

Synthesis of TBP

TBP was synthesized following the synthetic routes in the reported works.[1,2] The synthetic steps were listed in Scheme S1 and the procedures were described below.

Synthesis of **3**: (4-(7-bromobenzo[c][1,2,5]thiadiazol-4-yl)-N,N-diphenylaniline): A mixture of **1** (3 g, 10 mmol), **2** (2.6 g, 9 mmol), $Pd(PPh_3)_4$ (346 mg, 0.3 mmol) and K_2CO_3 (13.8 g, 100 mmol) in THF (100 mL) and water (20 mL) was heated at 80°C under nitrogen atmosphere for 8 h. After cooling to room temperature, the mixture was extracted with DCM for 3 times and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography with DCM and hexane (1:9, v/v) to afford the desired product as orange solid (2.07 g, 50 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 7.90-7.89 (1H, d), 7.82-7.79 (2H, d), 7.55-7.53 (1H, d), 7.32-7.28 (4H, m), 7.19-7.18 (6H, d), 7.09-7.06 (2H, t). Mass spectrum (MALDI-TOF), *m*/*z* calcd. for $C_{24}H_{16}BrN_3S_3$: 457.0248, found: 459.0185 $[M]^+$.

Synthesis of compound TBP (N,N-diphenyl-4-(7-(pyridin-4-yl)benzo[c][1,2,5]thiadiazol-4-yl)aniline): A mixture of **3** (1 g, 2.18 mmol), **4** (536 mg, 4.36 mmol), $Pd(PPh_3)_4$ (75 mg, 0.065 mmol) and K_2CO_3 (3 g, 21.8 mmol) in THF (50 mL) and water (10 mL) was heated at 80°C under nitrogen atmosphere for 8 h. After cooling to room temperature, the mixture was extracted with DCM for 3 times and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography with EA and DCM (1:99, v/v) to afford the desired product as red solid (776 mg, 78 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 8.78-8.76 (2H, q), 7.95-7.94 (2H, d), 7.94-7.87 (3H, d), 7.80-7.78 (1H, d), 7.32-7.28 (4H, t), 7.22-7.18 (6H, t), 7.10-7.06 (2H, t). ^{13}C NMR (400 MHz, $CDCl_3$): δ (TMS, ppm) 154.07, 153.63, 150.20, 148.51, 147.34, 144.74, 134.71, 130.17, 130.08, 129.44, 129.29, 128.97, 126.90, 125.10, 123.56, 123.50, 122.57. Mass spectrum (MALDI-TOF), *m*/*z* calcd. for $C_{29}H_{20}N_4S$: 456.1409, found: 456.1402 $[M]^+$.

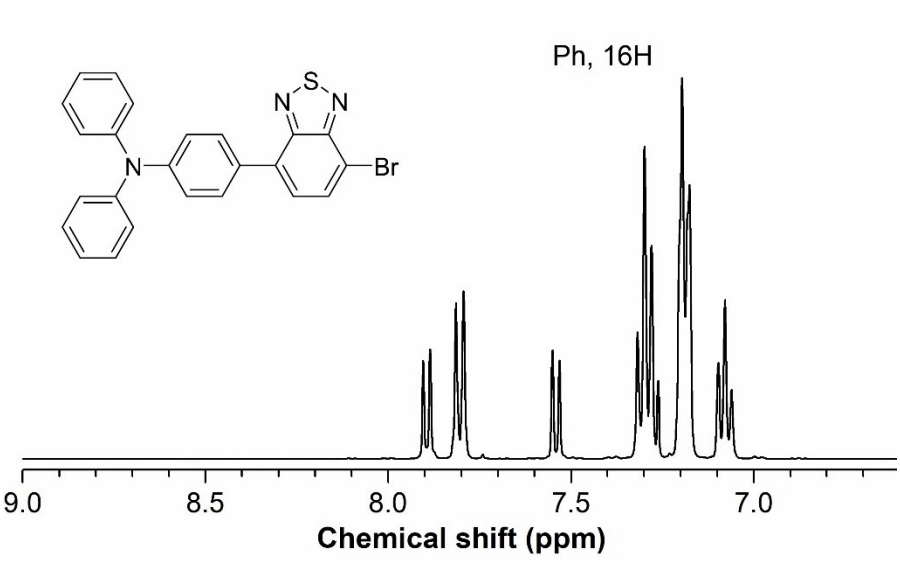

Figure S1. ^{1}H NMR spectrum of **3** in $CDCl_3$.

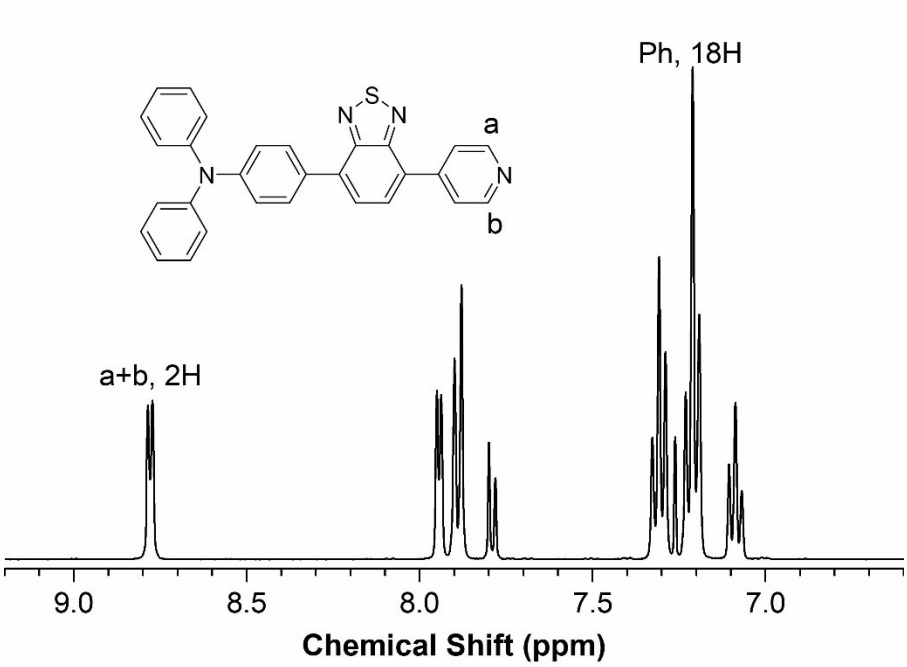

Figure S2. ^{1}H NMR spectrum of **TBP** in $CDCl_3$.

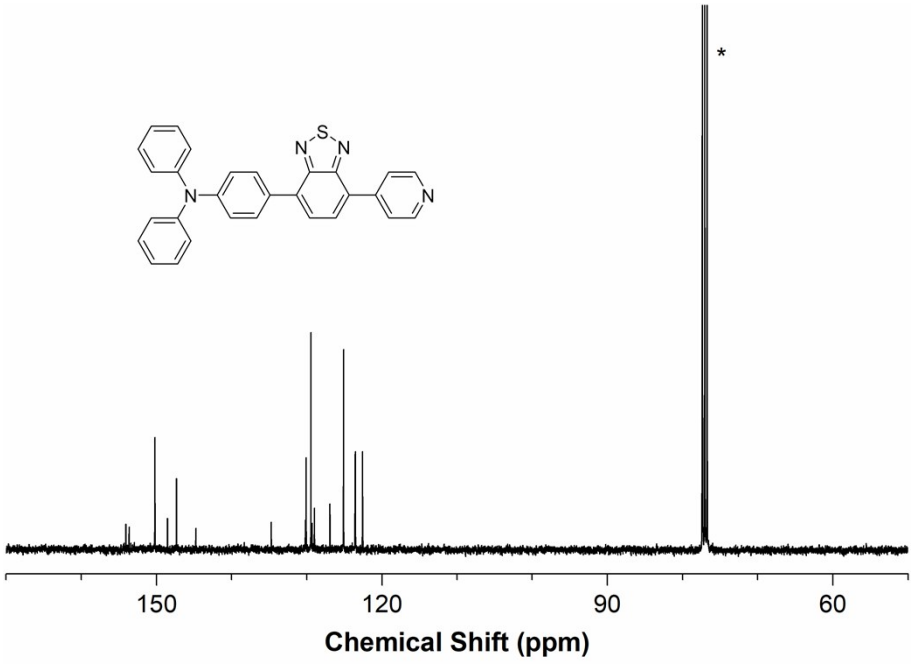

Figure S3. ^{13}C NMR spectrum of **TBP** in $CDCl_3$.

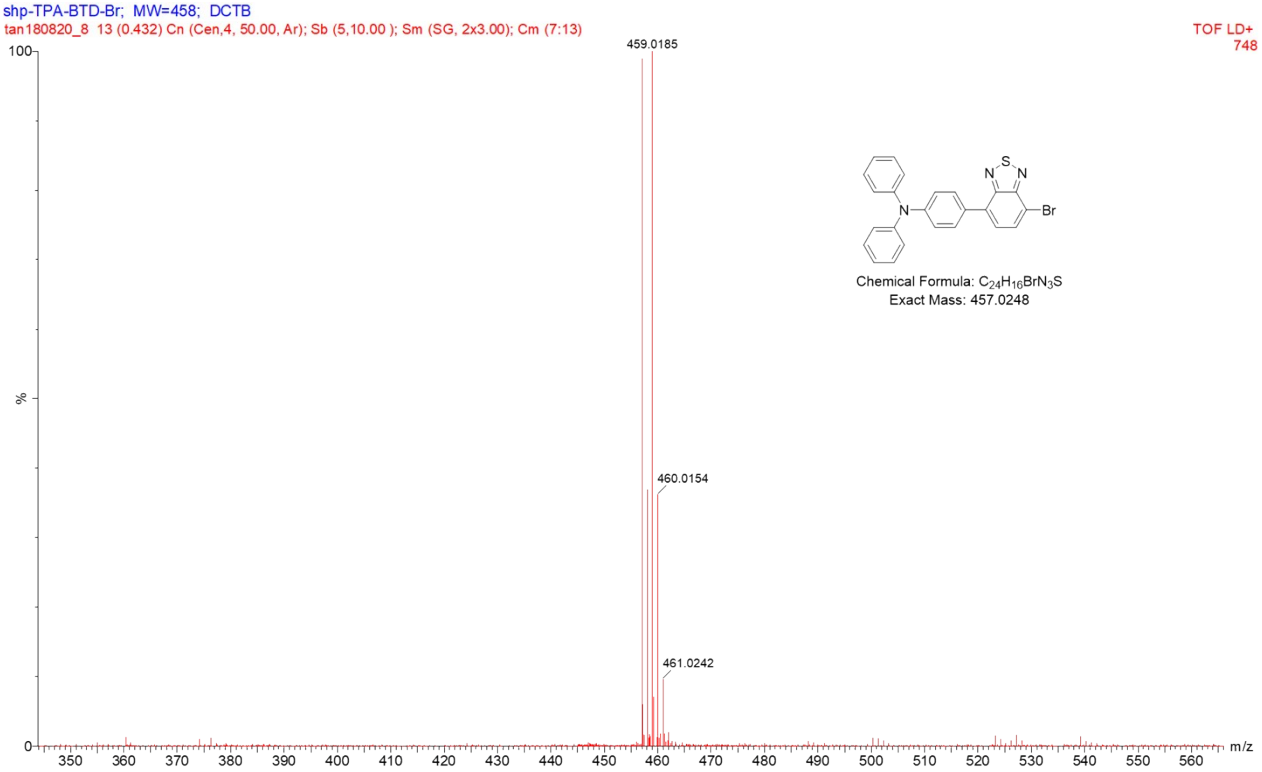

Figure S4. The high-resolution mass spectrum of **3.**

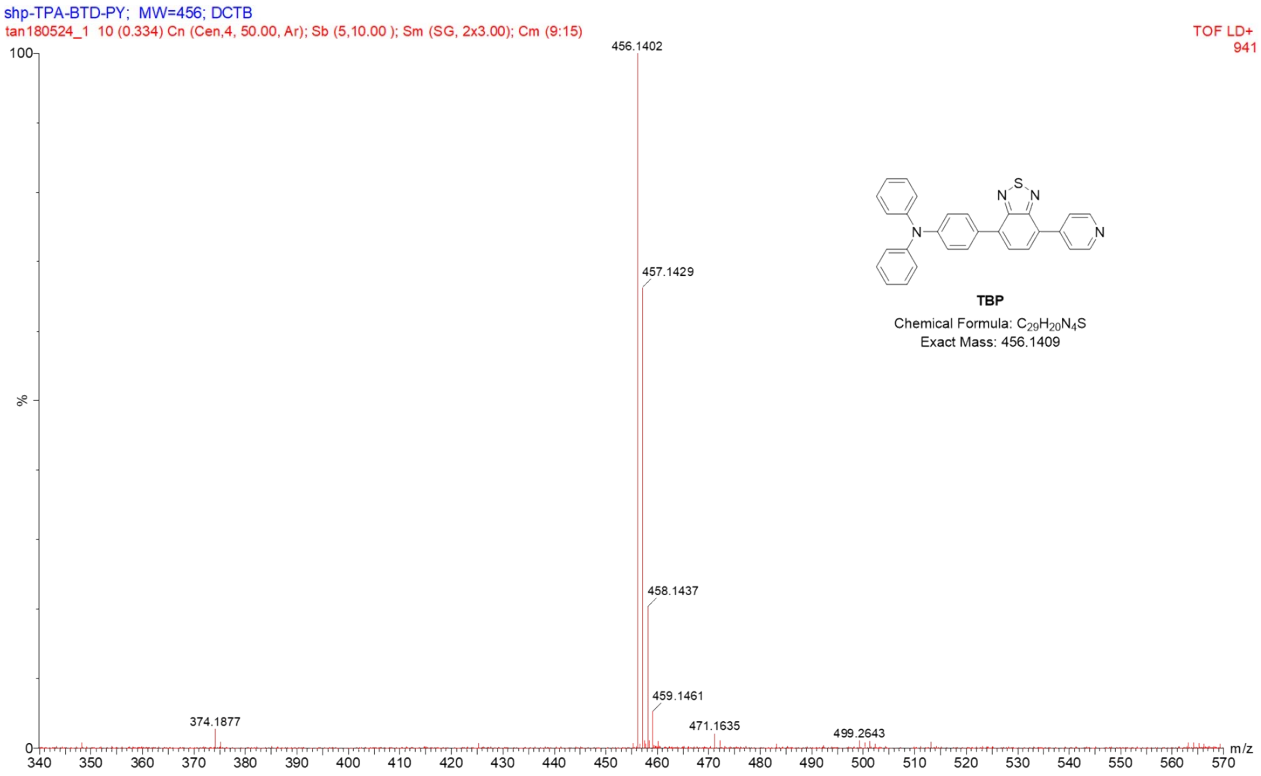

Figure S5. The high-resolution mass spectrum of **TBP**.

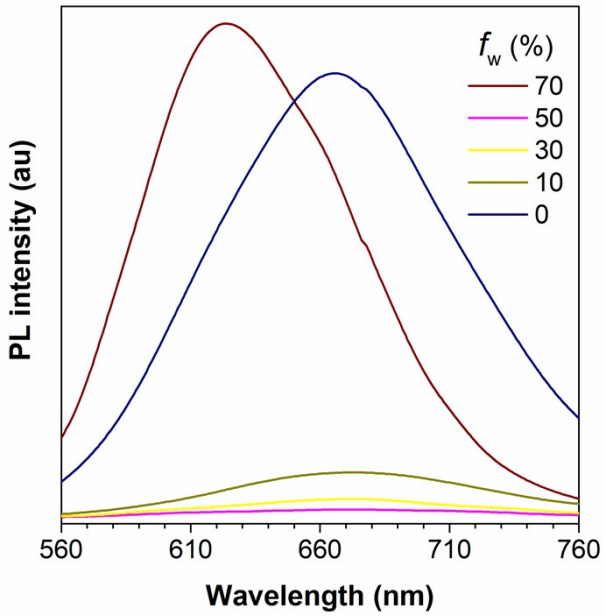

Figure S6. PL curves of TBP (10 μM) in ACN/water mixtures with different water fractions (f_w).

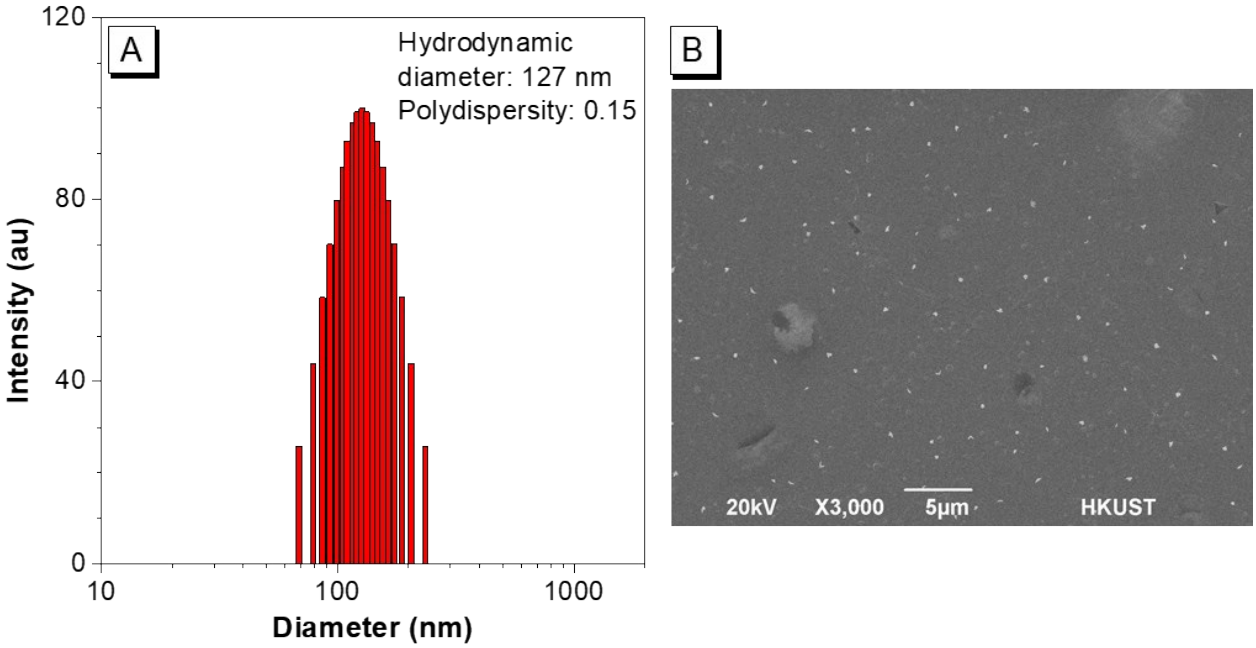

Figure S7. (A) Particle size distribution and (B) SEM image of TBP aggregates in the ACN/water mixture with a water fraction of 90%. SEM showed the aggregates with a diameter of about 120 nm.

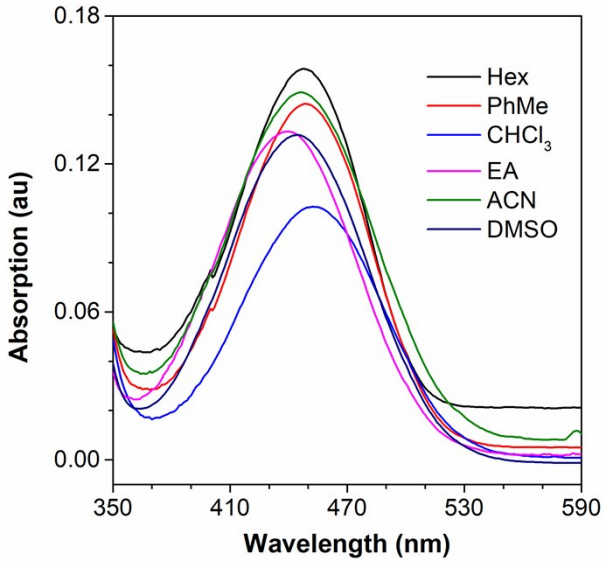

Figure S8. The UV-vis spectra of TBP in different solvents.

Table S1. Photophysical properties of TBP in different solvents.

Solvents	$E_T(30)$ [a] [kcal mol^{-1}]	λ_{abs} [nm]	λ_{em} [nm]	Stokes shift [nm]
Hex	31	448	526	78
PhMe	33.9	449	570	121
$CHCl_3$	39.1	453	615	162
EA	38.1	438	614	176
ACN	45.6	446	667	221
DMSO	45.1	444	671	227

a) The $E_T(30)$ of each solvent was collected from reference.[3]

Table S2. Optical properties of TBP.

AIEgen	λ_{abs} [nm] ($\varepsilon\times10^4$ M^{-1} cm^{-1}) [a]	λ_{em} [nm]			τ [ns][f]	k_r [ns^{-1}][f]	k_{nr} [ns^{-1}][f]
		Solution (φ_F)[b,c]	Aggregate (φ_F)[c,d]	Powder (φ_F)[c,e]			
TBP	444 (1.32)	671 (3.5%)	622 (30.2%)	600 (24.7%)	5.0	0.05	0.15

a) Absorption peak and the molar extinction coefficient of TBP in DMSO solutions; b) Emission peak in DMSO; c) Fluorescence quantum yield determined by a calibrated integrating sphere; d) Emission maximum in 99% PBS (containing 1% DMSO); e) Emission peak of TBP powder; f) TBP powder measured under ambient conditions. Radiative decay rate calculated using $k_r = \varphi_F/\tau$; Non-radiative decay rate calculated using $\varphi_F = k_r / (k_r + k_{nr})$.

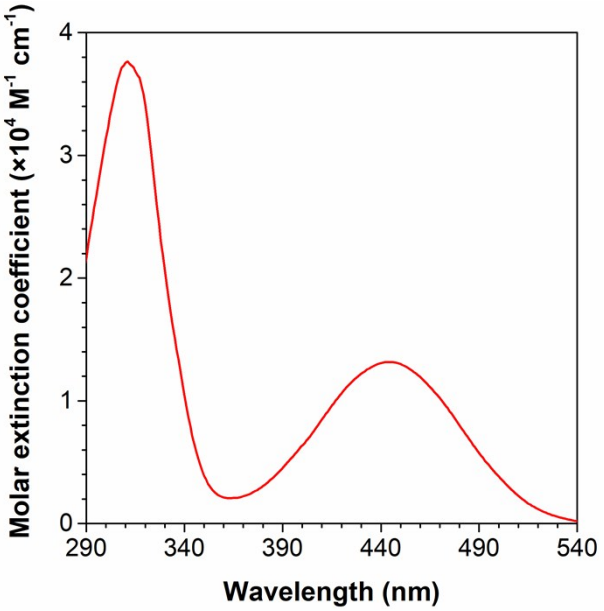

Figure S9. The absorption curve of TBP in DMSO.

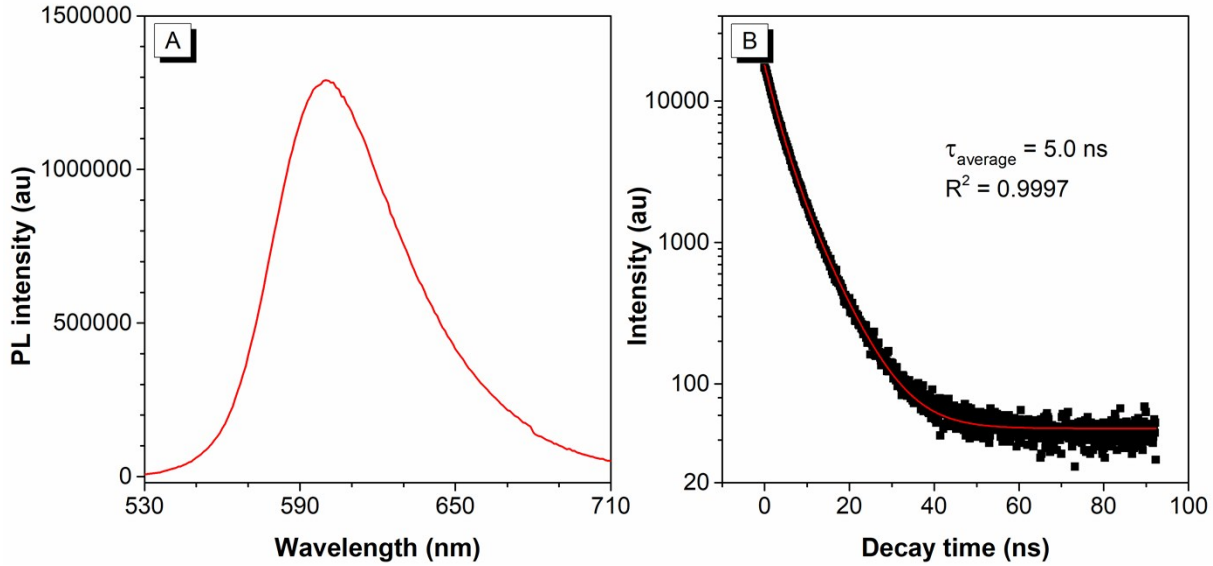

Figure S10. (A) The PL spectrum of TBP powder. (B) Fluorescence decay curve of TBP powder.

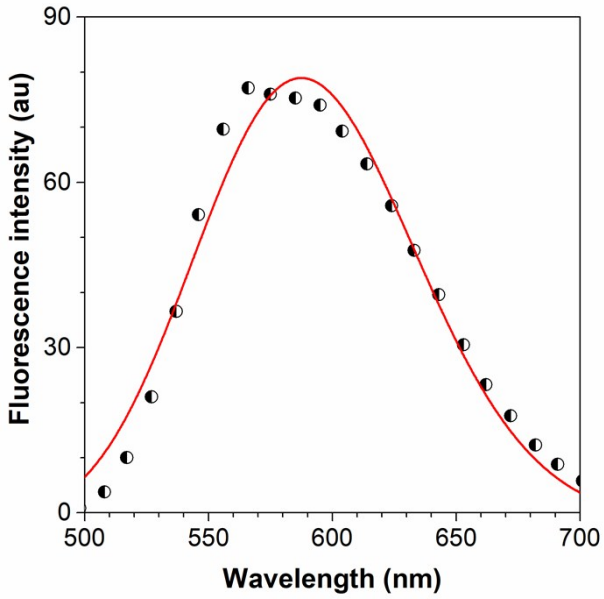

Figure S11. Fluorescence spectrum of HeLa cells stained with TBP. λ_{ex} = 488 nm.

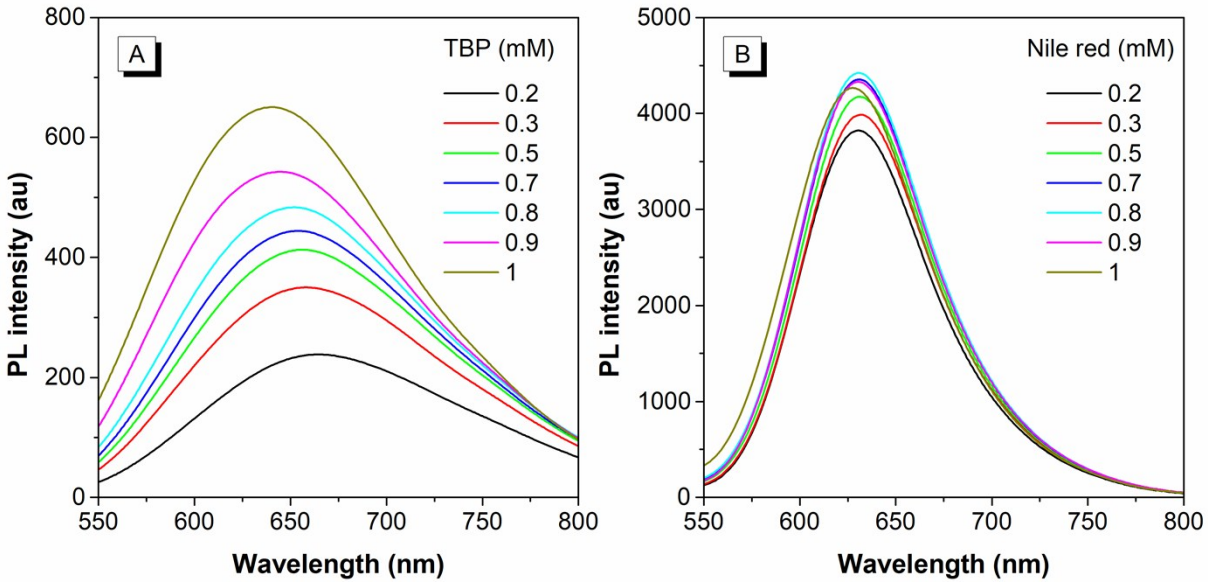

Figure S12. The fluorescence emission curves of (A) TBP or (B) Nile red with different concentrations in oleic acid solutions. Condition: for TBP, λ_{ex} = 430 nm; for Nile red, λ_{ex} = 530 nm.

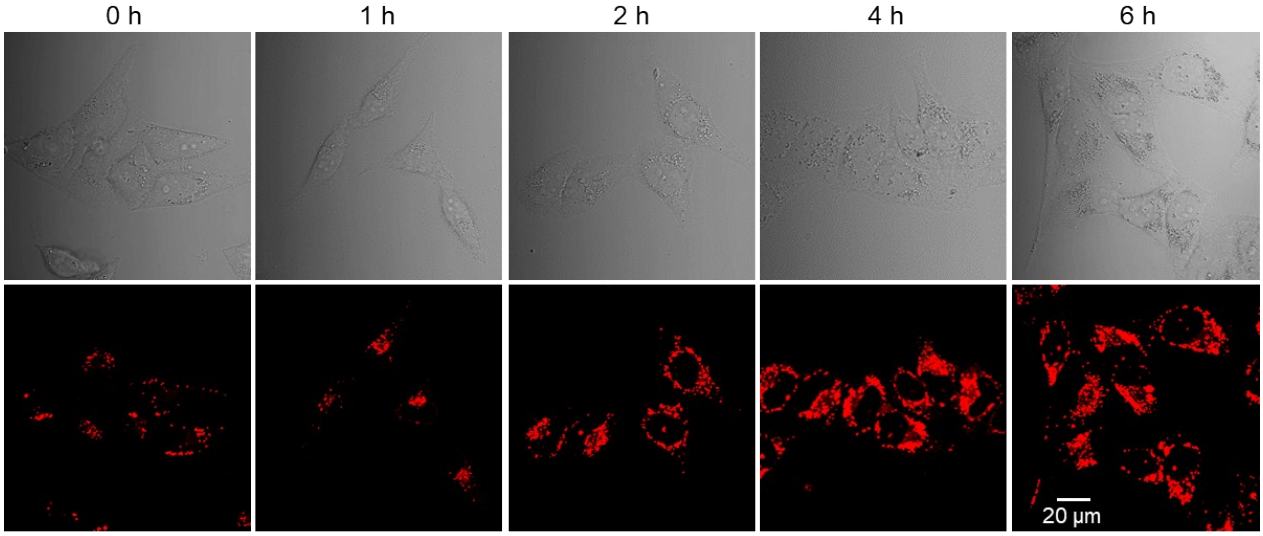

Figure S13. Confocal images of HeLa cells treated with 50 μM oleic acid for different times and then stained with 5 μM TBP for 30 min. Condition: λ_{ex} = 488 nm, λ_{em} = 550–740 nm. Scale bar: 20 μm.

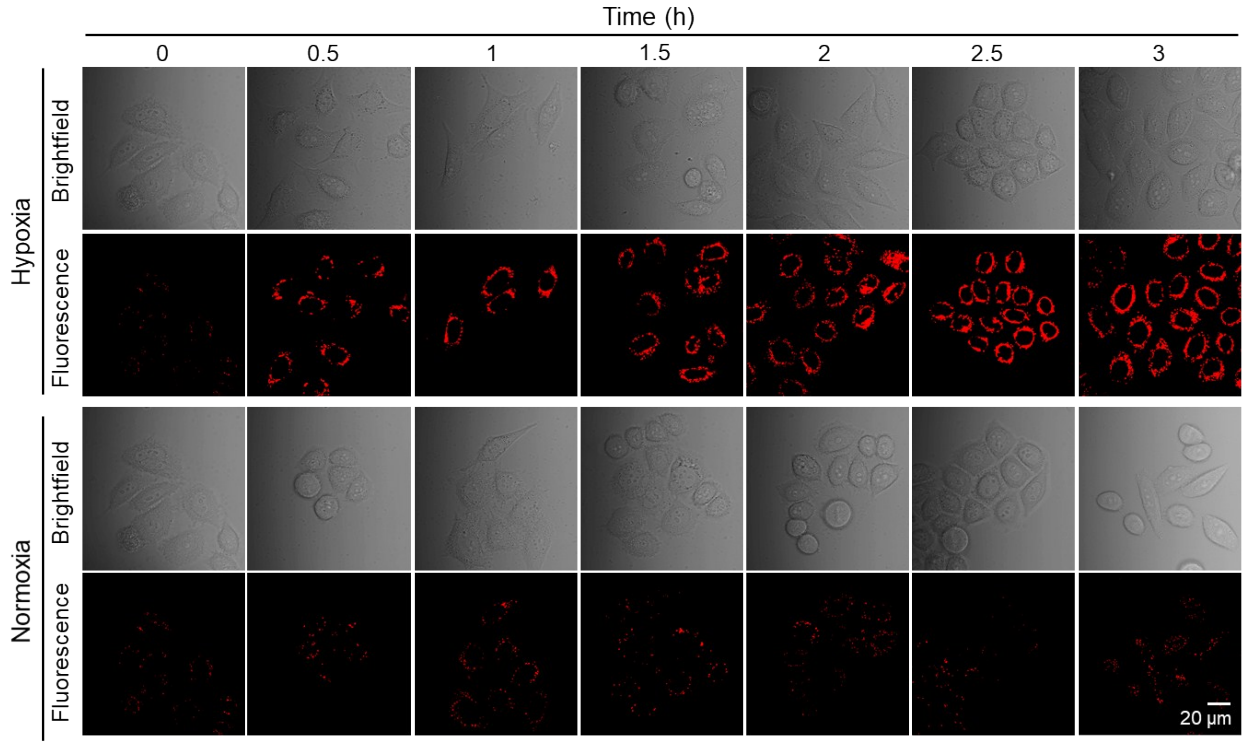

Figure S14. Confocal images of HepG2 cells under hypoxic or normoxic condition for different times, then incubated with 5 μM TBP for 30 min. Condition: λ_{ex} = 488 nm, λ_{em} = 550–740 nm; Scale bar: 20 μm.

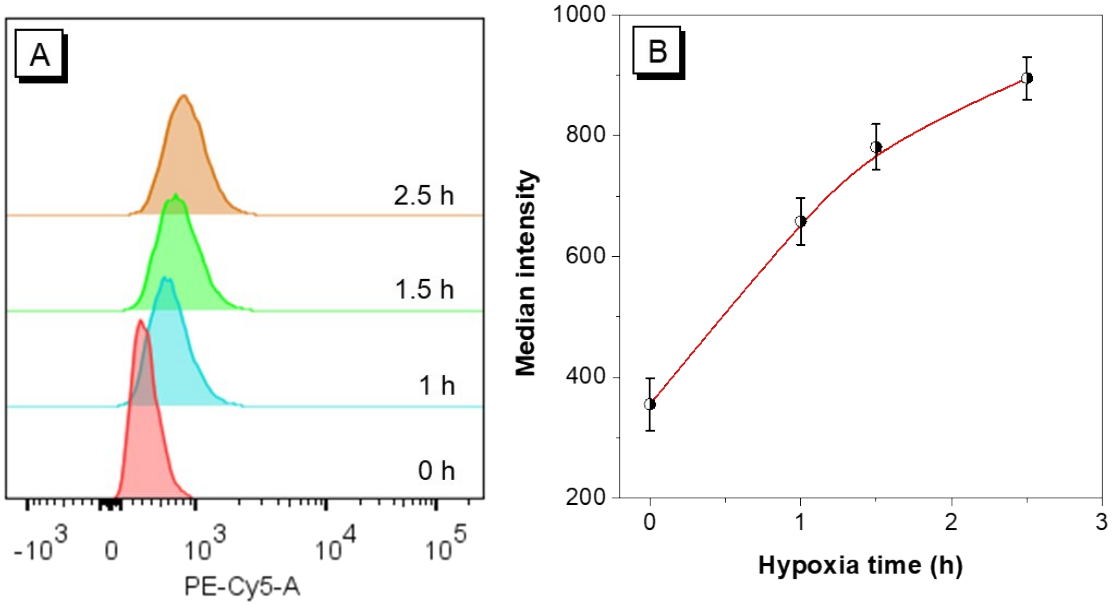

Figure S15. (A) The flow cytometry results of HepG2 cells in hypoxic environment for different times, then incubated with 5 μM TBP for 30 min. (B) The median fluorescence intensity of HepG2 cells in hypoxic environment for different times acquired from the flow cytometry results in A. Condition, λ_{ex} = 488 nm, λ_{em} = 655±15 nm.

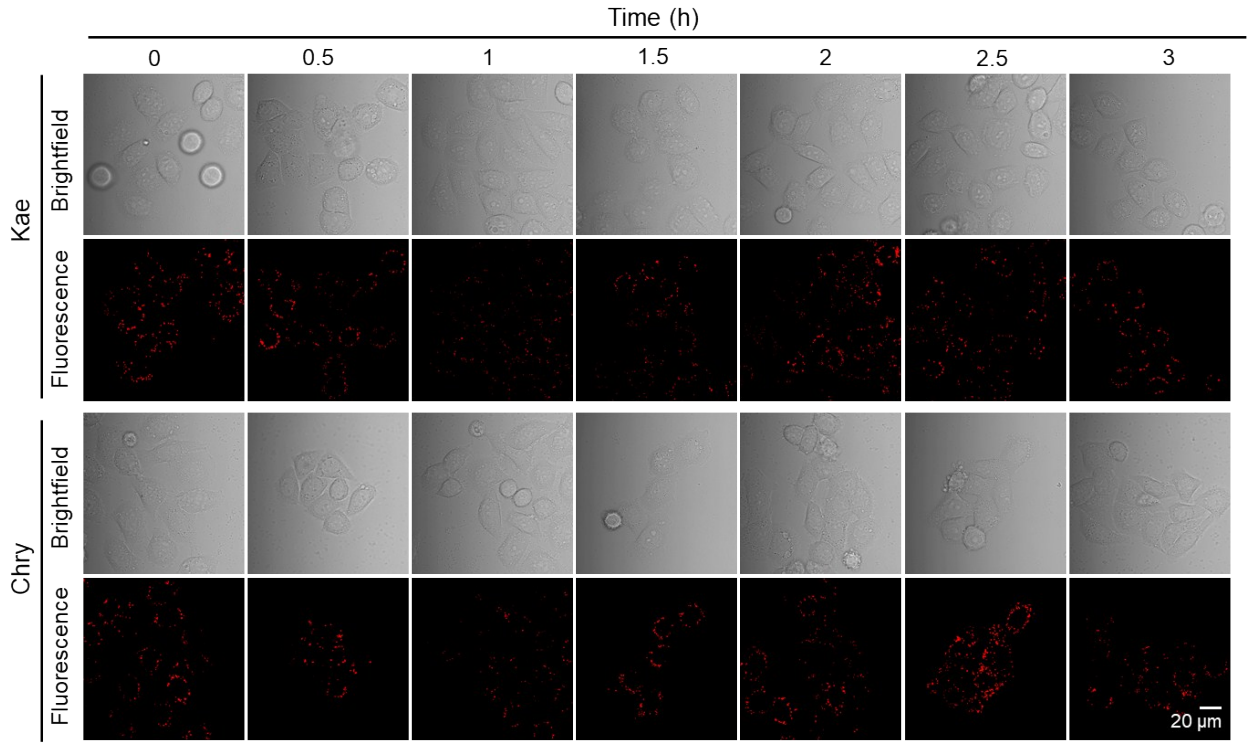

Figure S16. Confocal images of HepG2 cells pre-treated with 50 μM Kae or Chry for 30 min followed by staying in hypoxia environment for different times, and then incubated with 5 μM TBP for 30 min. Condition: λ_{ex} = 488 nm, λ_{em} = 550–740 nm; Scale bar: 20 μm.

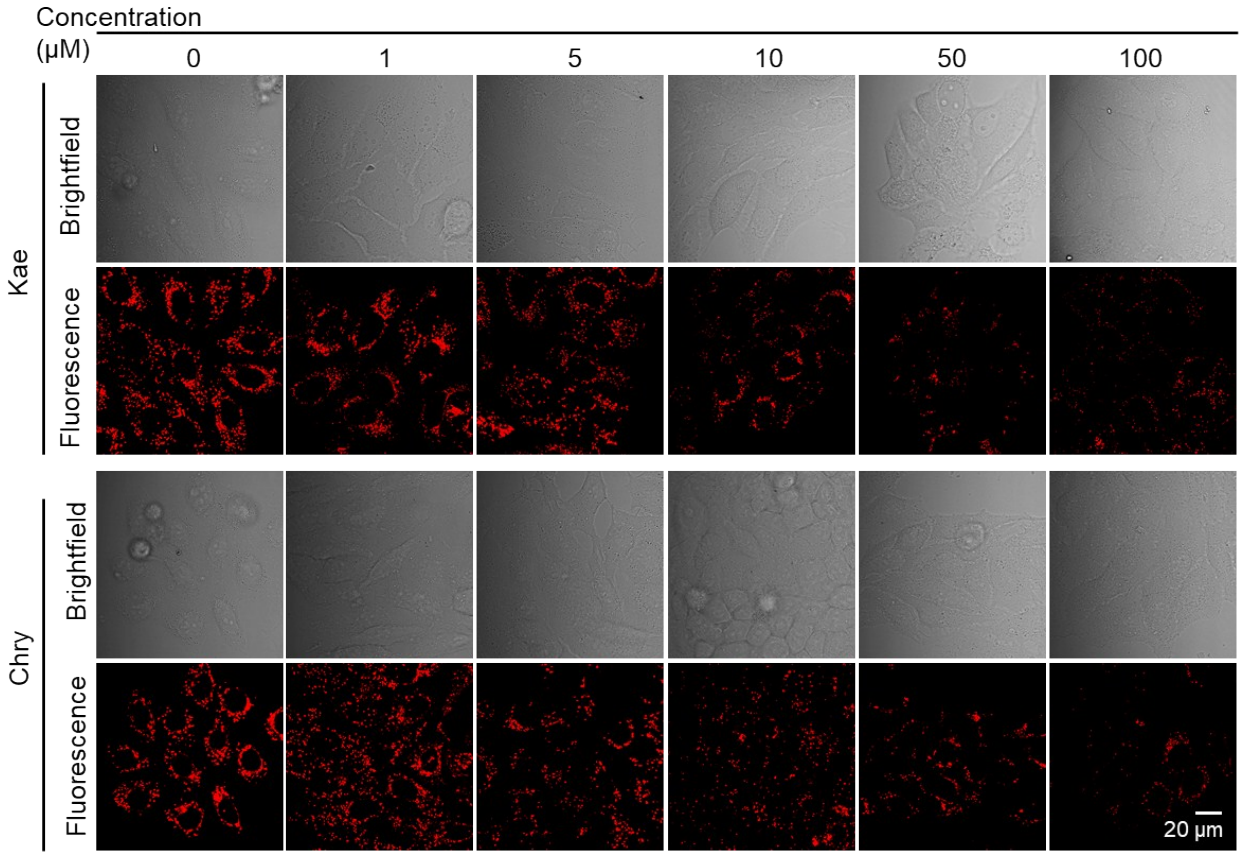

Figure S17. Confocal images of HepG2 cells pre-treated with different concentration of Kae or Chry for 30 min followed by staying in hypoxic environment for 3 h, then incubated with 5 μM TBP for 30 min. Condition: λ_{ex} = 488 nm, λ_{em} = 550–740 nm. Scale bar: 20 μm.

Table S2. Crystal data and structure refinement for **TBP**.

Bond precision:	C-C = 0.0019 Å		Wavelength=1.54184
Cell:	a=9.66643(15)	b=9.81044(16)	c=23.5812(4)
	alpha=90	beta=98.0470(16)	gamma=90
Temperature:	100 K		
	Calculated		Reported
Volume	2214.23(6)		2214.23(6)
Space group	P 21/n		P 1 21/n 1
Hall group	-P 2yn		-P 2yn
Moiety formula	C29 H20 N4 S		C29 H20 N4 S
Sum formula	C29 H20 N4 S		C29 H20 N4 S
Mr	456.55		456.55
Dx, g cm^{-3}	1.370		1.370
Z	4		4
Mu (mm^{-1})	1.496		1.496
F000	952.0		952.0
F000'	955.75		
h,k,l_{max}	11,11,28		11,11,28
N_{ref}	3992		3980
T_{min},T_{max}	0.806, 0.956		0.861, 1.000
$T_{min'}$	0.799		

Correction method= # Reported T Limits: T_{min}=0.861 T_{max}=1.000 AbsCorr = MULTI-SCAN

Data completeness= 0.997	Theta(max)= 67.486
R(reflections)= 0.0303(3556)	wR2(reflections)= 0.0794(3980)
S = 1.030	Npar= 307
CCDC	1945204

References

1. J. Mao, D. Wang, S.-H. Liu, Y. Hang, Y. Xu, Q. Zhang, W. Wu, P.-T. Chou and J. Hua, *Asian J. Org. Chem.*, 2014, **3**, 153-160.

2. L. Wang, X. Yang, J. Zhao, F. Zhang, X. Wang and L. Sun, *ChemSusChem*, 2014, **7**, 2640-2646.

3. C. R., *Chem. Rev.*, 1994, **21**, 2319.