A fluorescence turn-on probe for human (bovine) serum album based on the hydrolysis of dioxaborine group promoted by the proteins

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Supporting Information

Contents

Experiment

1 Materials and Reagents

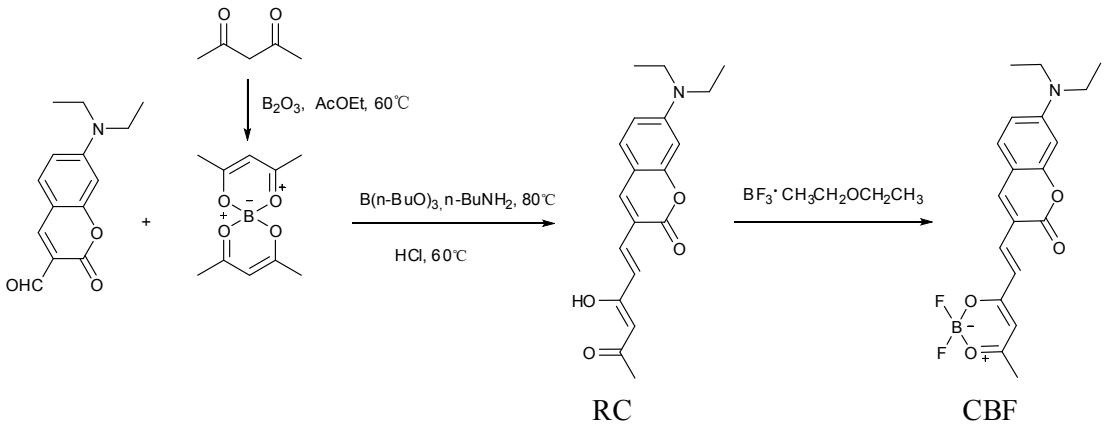
All chemicals were purchased from Aladdin Corporation and used without further purification. Ultra-pure water was prepared with a Sartorius Arium 611DI system.

2 Spectral measurements

Stock solution of the CBF (3.0×10^{-3} M in DMSO), bovine serum albumin (BSA, 36.0 mg/mL) and human serum albumin (HSA, 36.0 mg/mL) in PBS (20 mM, pH 7.4) were prepared in advance. The stock solution of CBF was diluted with corresponding solvents to acquire 5.0 μM dye solution. Absorption spectra were measured with an Evolution 220 UV-visible spectrophotometer (Thermo Scientific). Fluorescent spectra were carried out in a Lumina Fluorescence Spectrometer (Thermo Scientific). NMR spectra were performed with a Bruker AV-400 spectrometer (400M Hz). Mass spectra were recorded on a MA 1212 Instrument using standard condition (ESI, 70 ev).

3 Synthesis

Probe CBF was synthesized according to following procedures (Scheme 1).



Scheme S1 The synthesis procedures of CBF

The synthesis of RC: Acetyl acetone (2.00 g, 19.2 mmol) and boron trioxide (1.04 g, 14.9 mmol) were added into 5 mL ethyl acetate and stirred at 60 °C for 30 min under the protection of nitrogen. To the above solution, tributyl borate (4.60 g, 20.0 mmol, dissolved in 3 mL ethyl acetate) and 7-(diethylamino)-3-carbaldehyde-coumarine (2.45 g, 10.0 mmol, dissolved in 5 mL ethyl acetate) were dropped gradually, and the mixture was stirred for another 30 min at 80 °C. Then, *n*-butylamine (0.29 g, 0.40 mmol) dissolved in 1 mL ethyl acetate was added dropwise, and the reaction mixture was further stirred at 80 °C for 2.5 h followed by adding 20 mL HCl (1.0 mol/L) and stirred for another 30 min. Then the reaction solution was extracted with CH_2Cl_2 and the organic solvent was removed under reduced pressure. The residue was purified by column chromatography to give an orange solid (1.00 g, 35.6%). ^{1}H NMR (400 MHz, CDCl3) δ: 15.35 (s, 1H), 7.67 (s, 1H), 7.41 (d, J = 15.6 Hz, 1H), 7.30 (d, J = 8.9 Hz, 1H), 7.11 (d, J = 15.6 Hz, 1H), 6.60 (dd, J1 = 8.9 Hz, J2 = 2.5 Hz 1H), 6.49 (d, J = 2.4 Hz, 1H), 5.67 (s, 1H), 3.44 (q, J = 7.1 Hz, 4H), 2.16 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H). ^{13}C NMR (400 MHz, CDCl3) δ: 197.8, 176.9, 159.7, 156.1, 151.6, 144.7, 135.0, 130.4, 122.3, 113.3, 109.8, 108.3, 101.2, 96.2, 44.3, 26.7, 12.3. HR-MS m/z: 328.1530 $(M+H)^+$; calculated molecular weight of $C_{19}H_{21}NO_4$: 328.1549 for $(M+H)^+$ (Fig. S2).

The synthesis of compound CBF: To a stirring solution of RC (500 mg, 1.53 mmol) dissolved in 150 mL dehydrated dichloromethane, boron trifluoride-diethyl etherate (433 mg, 3.05 mmol) was added dropwise at room temperature. After stirring for 3 h, the reaction mixture was washed with saturated brines (100 mL ×3). The organic solvent was removed under reduced pressure. The residue was purified by column chromatography to give a dark purple solid (340 mg, 59.3%). ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.45 (s, 1H), 7.91 (d, J = 15.4 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 7.21 (d, J = 15.4 Hz, 1H), 6.84 (dd, J1 = 9.0 Hz, J2 = 1.4 Hz, 1H), 6.62 (s, 1H), 6.46 (s, 1H), 3.52 (q, J = 6.9 Hz, 4H), 2.33 (s, 3H), 1.56 (t, J = 6.9 Hz, 6H). ^{13}C NMR (400 MHz, DMSO-d6) δ: 190.2, 180.0, 159.3, 157.0, 153.0, 149.0, 144.1, 131.6, 118.4, 112.2, 110.5, 108.8, 101.4, 96.3, 44.5, 23.8, 12.4. ^{19}F NMR (400 MHz, DMSO-d6) δ: ^{10}BF: -136.74, ^{11}BF: -136.81. ^{11}B NMR (400 MHz, DMSO-d6) δ: 1.04. HR-MS m/z: 375.1451 $(M+H)^{+}$; calculated molecular weight of $C_{19}H_{20}BF_2NO_4$: 375.1453 for $(M+H)^{+}$ (Fig. S1).

4 HSA/BSA Titrations

5.0 μL of the dye stock solution were added to 3.0 mL of phosphate buffer solution (20 mM, pH 7.4) to keep [CBF] = 5.00 μM. 0 ~ 100 μL of 36.0 mg/mL HSA/BSA in PBS were added into the above solution to obtain appropriate concentrations of HSA/BSA. The absorption and emission spectra were recorded 10 min after the addition of HSA (or 30 min for BSA).

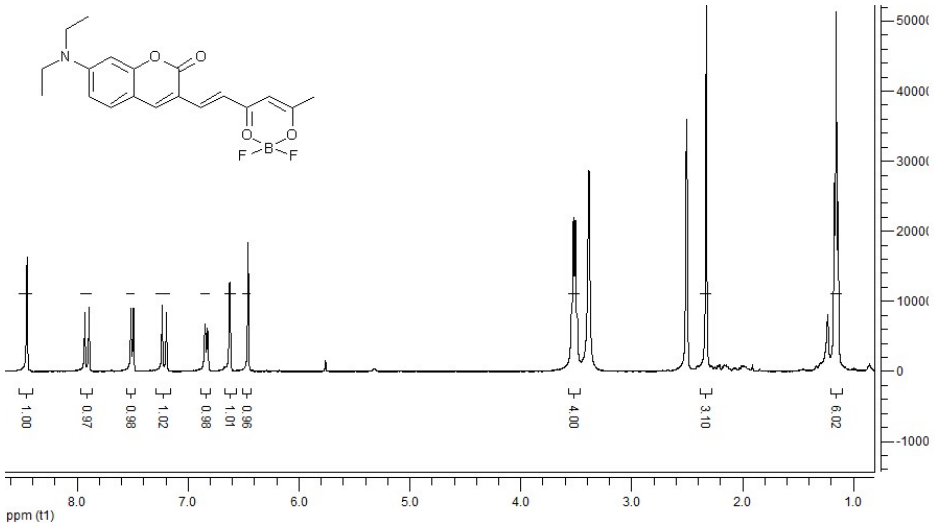
5 Competition experiment

HSA (0.50 mg/mL) was mixed with various concentrations of drug (warfarin or ibuprofen) in PBS (20 mM, pH 7.4) for 30 min. Then 5.0 μL of the dye stock solution were added into above solution to keep [CBF] = 5 μM.

HSA (0.50 mg/mL) was mixed with CBF (5.0 μM) in PBS (20 mM, pH 7.4) for 30 min. Then, 0 – 40 μL of warfarin/ibuprofen stock solution (15 mM in DMSO) were added into the above solution to obtain various concentrations of warfarin/ibuprofen. The emission spectra were recorded after mixed for 30 min.

6 Molecular docking

Docking simulations were carried out by using CDOCKER module (Discovery Studio, version 2.1, Accelrys, San Diego, CA, USA). The X-ray crystal structures of HSA complexed with phenylbutazone (PDB ID: 2BXP) or ibuprofen (PDB ID: 2BXG) were used for the docking calculation. After removing the ligands and solvent molecules, the CHARMm-force field was applied to the protein. The area around phenylbutazone and ibuprofen was defined as binding site I and site II, respectively, with a radius set as 7.5 Å. Random conformations of compounds CBF and RC were generated using CHARMm-based molecular dynamics (1000 steps), and then docked into the defined binding sites. The other parameters were set as default. The final binding conformations of compounds CBF and RC were determined based on the calculated CDOCKING energy and visual check. The most stable binding mode among the top 10 docking poses of each compound was presented in Fig. S8.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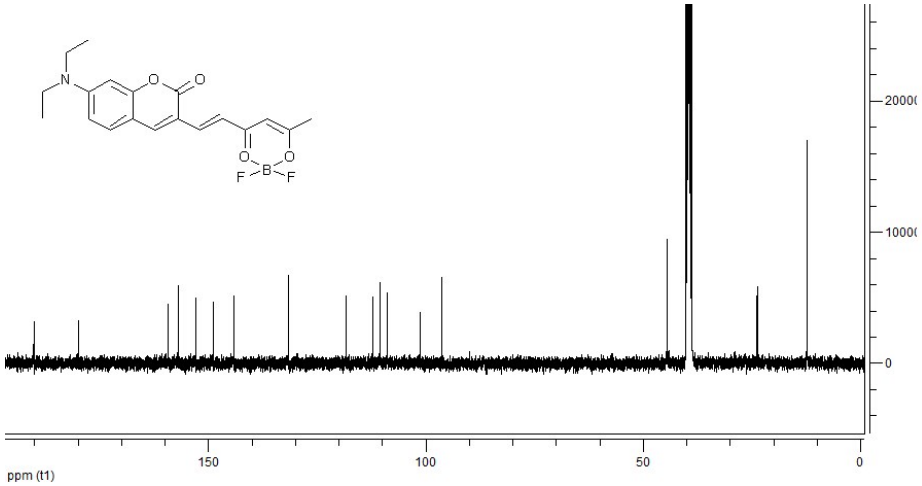
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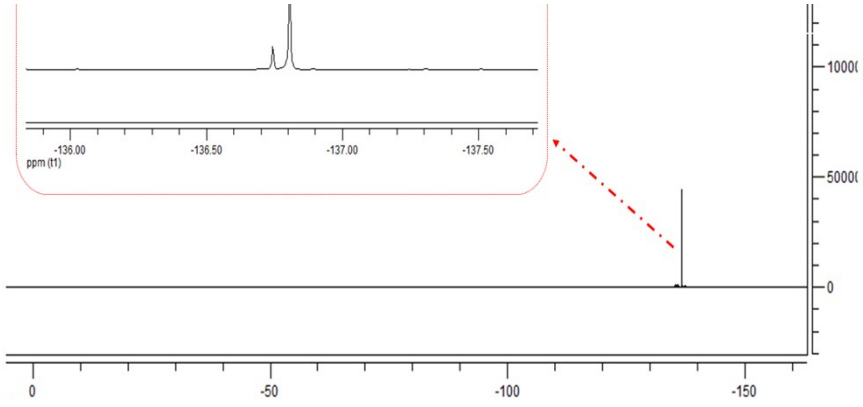
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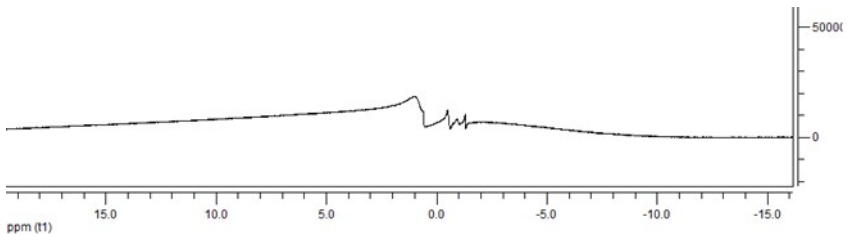
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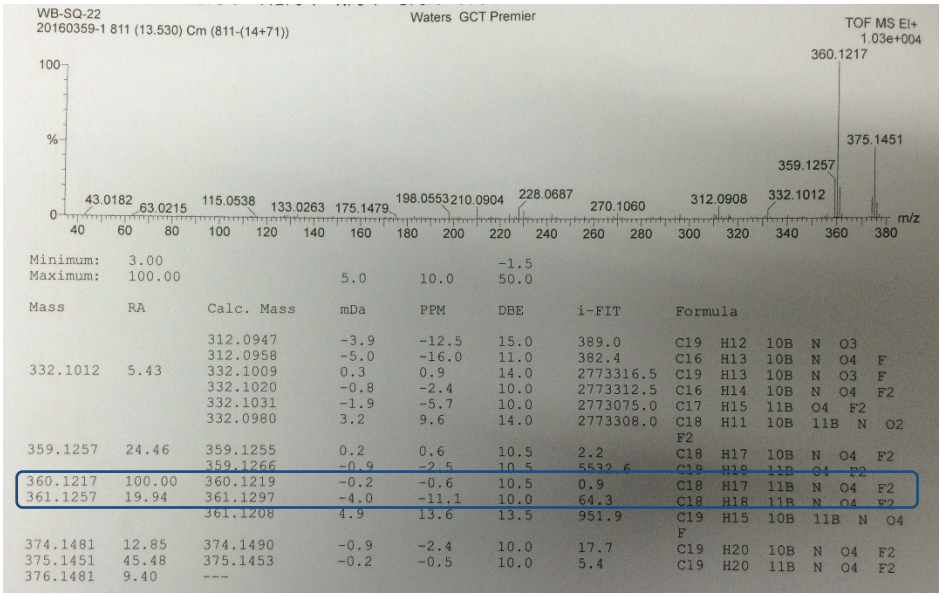
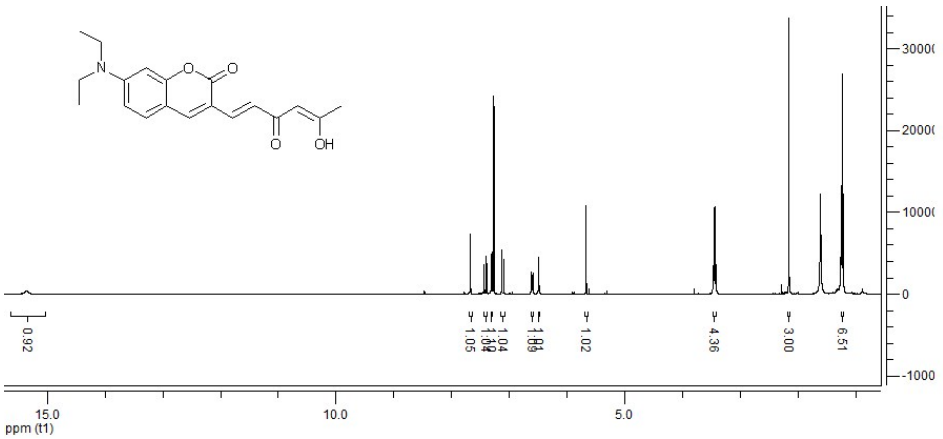
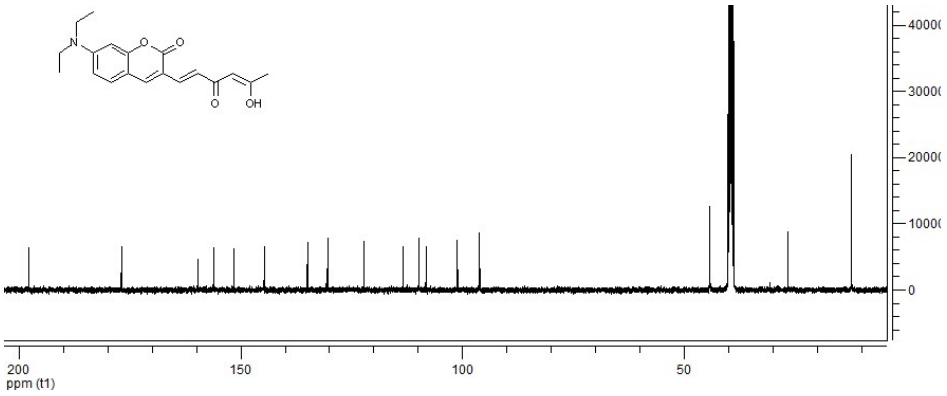


Minimum:	3.00				-1.5		
Maximum:	100.00		5.0	10.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
		312.0947	-3.9	-12.5	15.0	389.0	C19 H12 10B N O3
		312.0958	-5.0	-16.0	11.0	382.4	C16 H13 10B N O4 F
332.1012	5.43	332.1009	0.3	0.9	14.0	2773316.5	C19 H13 10B N O3 F
		332.1020	-0.8	-2.4	10.0	2773312.5	C16 H14 10B N O4 F2
		332.1031	-1.9	-5.7	10.0	2773075.0	C17 H15 11B O4 F2
		332.0980	3.2	9.6	14.0	2773308.0	C18 H11 10B 11B N O2 F2
359.1257	24.46	359.1255	0.2	0.6	10.5	2.2	C18 H17 10B N O4 F2
		359.1266	-0.9	-2.5	10.5	5532.6	C19 H18 11B O4 F2
360.1217	100.00	360.1219	-0.2	-0.6	10.5	0.9	C18 H17 11B N O4 F2
361.1257	19.94	361.1297	-4.0	-11.1	10.0	64.3	C18 H18 11B N O4 F2
		361.1208	4.9	13.6	13.5	951.9	C19 H15 10B 11B N O4 F
374.1481	12.85	374.1490	-0.9	-2.4	10.0	17.7	C19 H20 10B N O4 F2
375.1451	45.48	375.1453	-0.2	-0.5	10.0	5.4	C19 H20 11B N O4 F2
376.1481	9.40	---					

Fig. S1 The ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR and ^{11}B-NMR and HR-MS spectra of CBF.





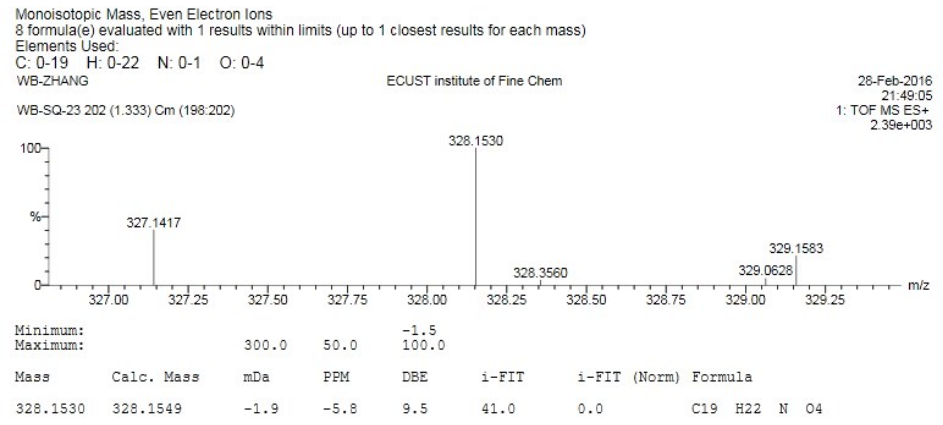


Fig. S2 The ^{1}H-NMR, ^{13}C-NMR and HR-MS spectra of RC.

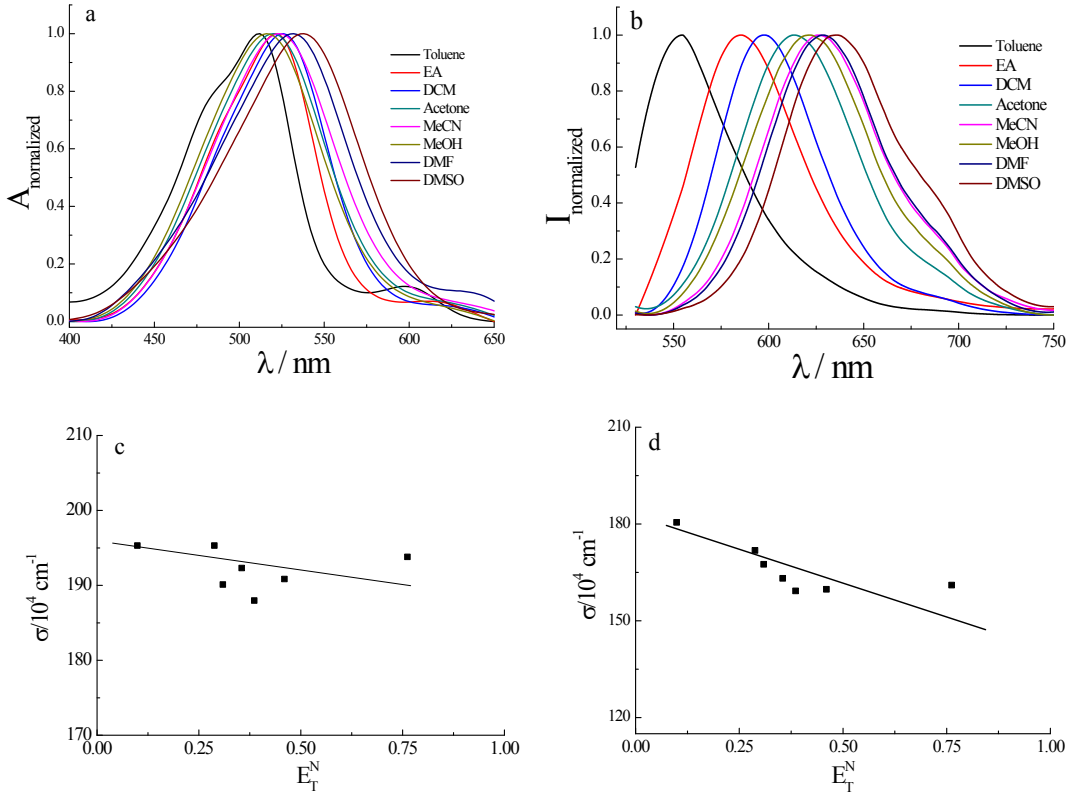


Fig. S3 Normalized absorption (a) and emission (b) spectra of CBF in various solvents, and the correlation of absorption (c) and emission (d) wavenumber of CBF with solvent polarity. Recorded 5 min after the addition of the probe.

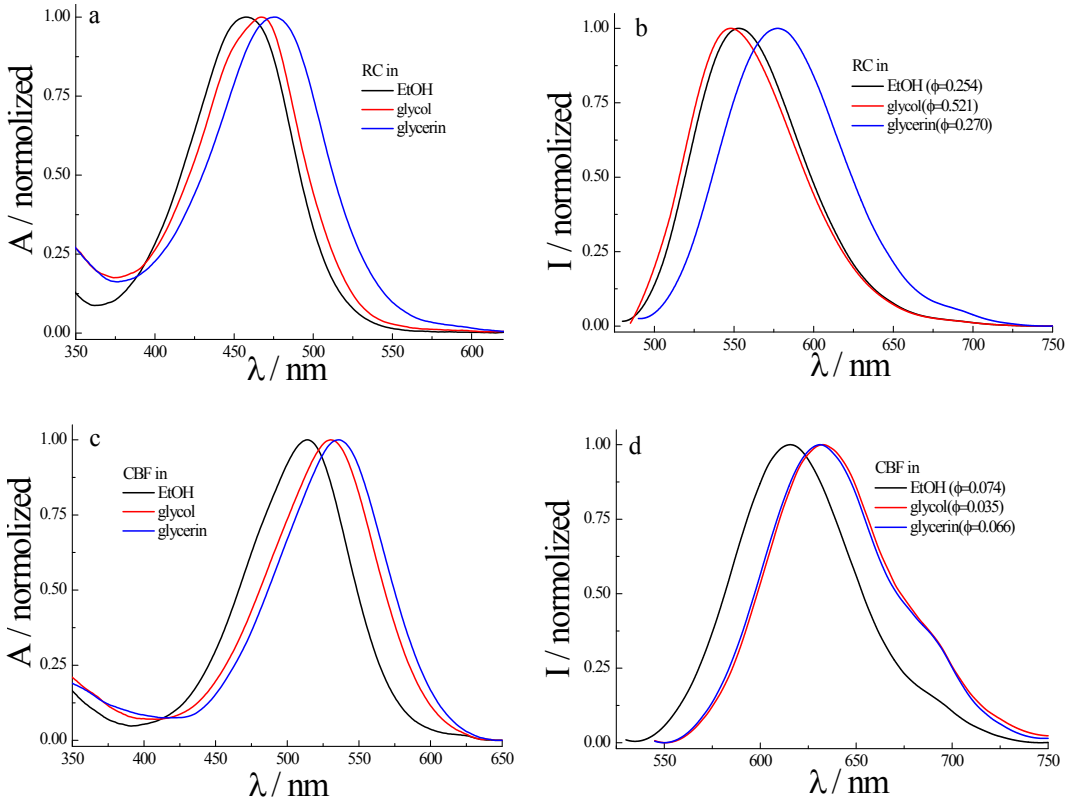


Fig. S4 Normalized absorption (a, c) and emission (b, d) spectra of RC (a-b) and CBF (c-d) in solvents with different viscosities. Recorded 5 min after the addition of the probe.

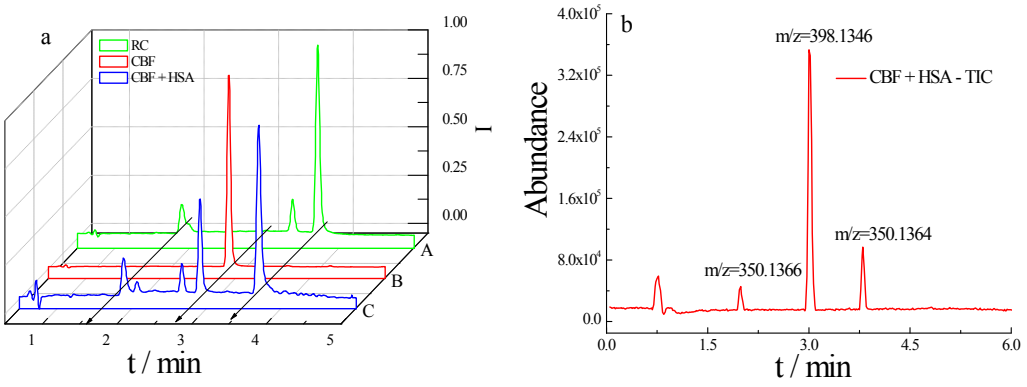


Fig. S5 (a) The UPLC of RC (green), CBF (red) and the solution of CBF with HSA (blue) in PBS; (b) The total ion chromatogram of the mixture of CBF and HSA in PBS after 5 min. [CBF] = [RC] = 5.0 μM, [HSA] = 1.0 mg/mL, 20 mM PBS (pH 7.4), detection wavelength = 460 nm.

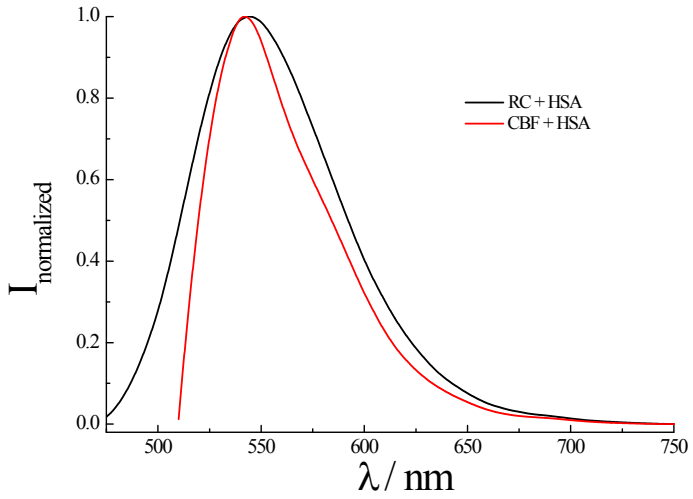


Fig. S6 Normalized emission spectra of RC (black) and CBF (red) mixed with HSA for 15 min in PBS. [RC] = [CBF] = 5.0 μM, [HSA] = 1.0 mg/mL, 20 mM PBS (pH 7.4), λ_{ex} = 495 nm.

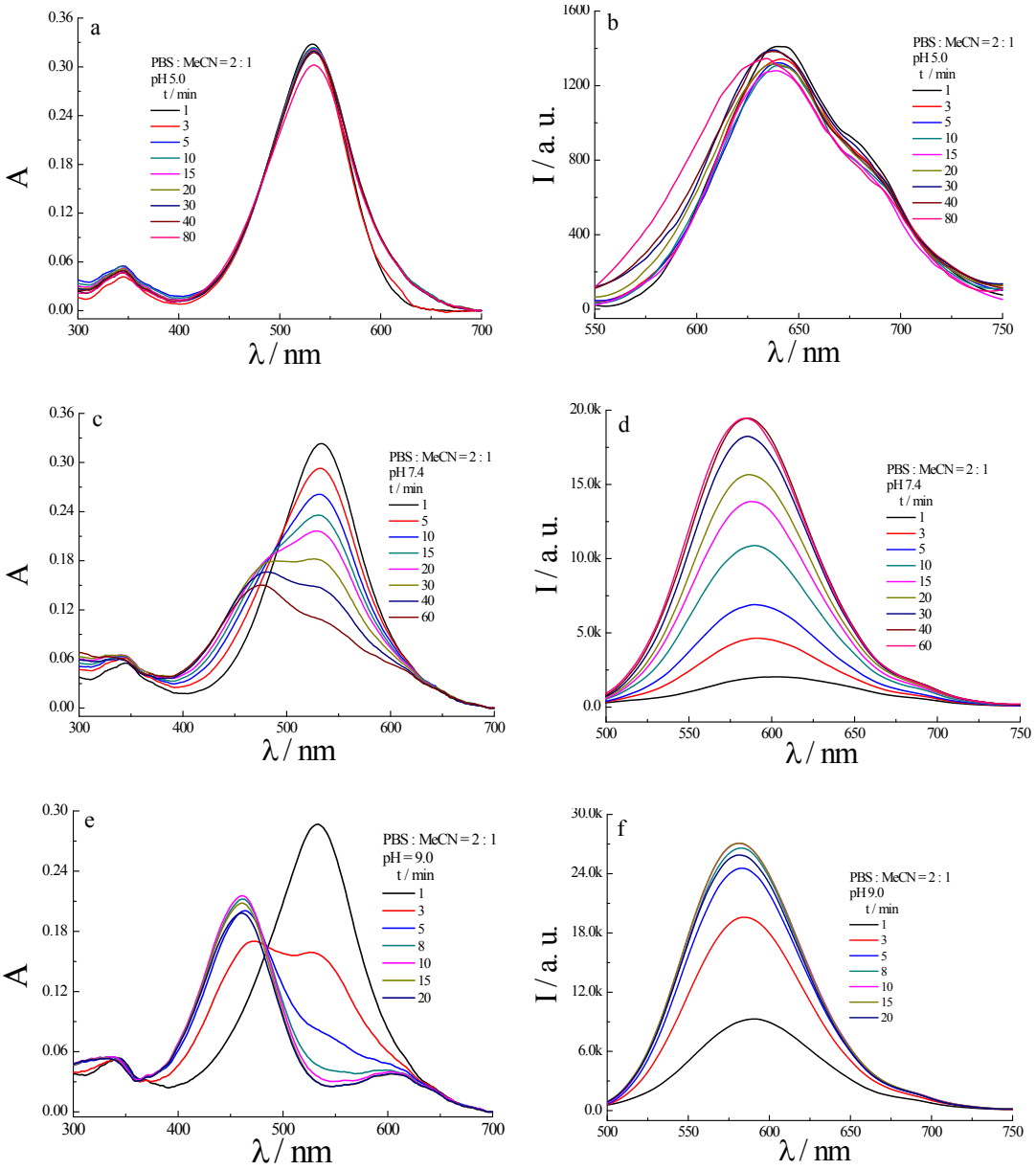


Fig. S7 Time-dependent absorption (a, c, e) and emission (b, d, f) spectra of CBF in mixed solution of PBS: MeCN (v/v = 2:1) with different pHs. (a-b) pH 5.0; (c-d) pH 7.4; (e-f) pH 9.0. [CBF] = 5.0 μM, 20 mM, λ_{ex} = 495 nm.

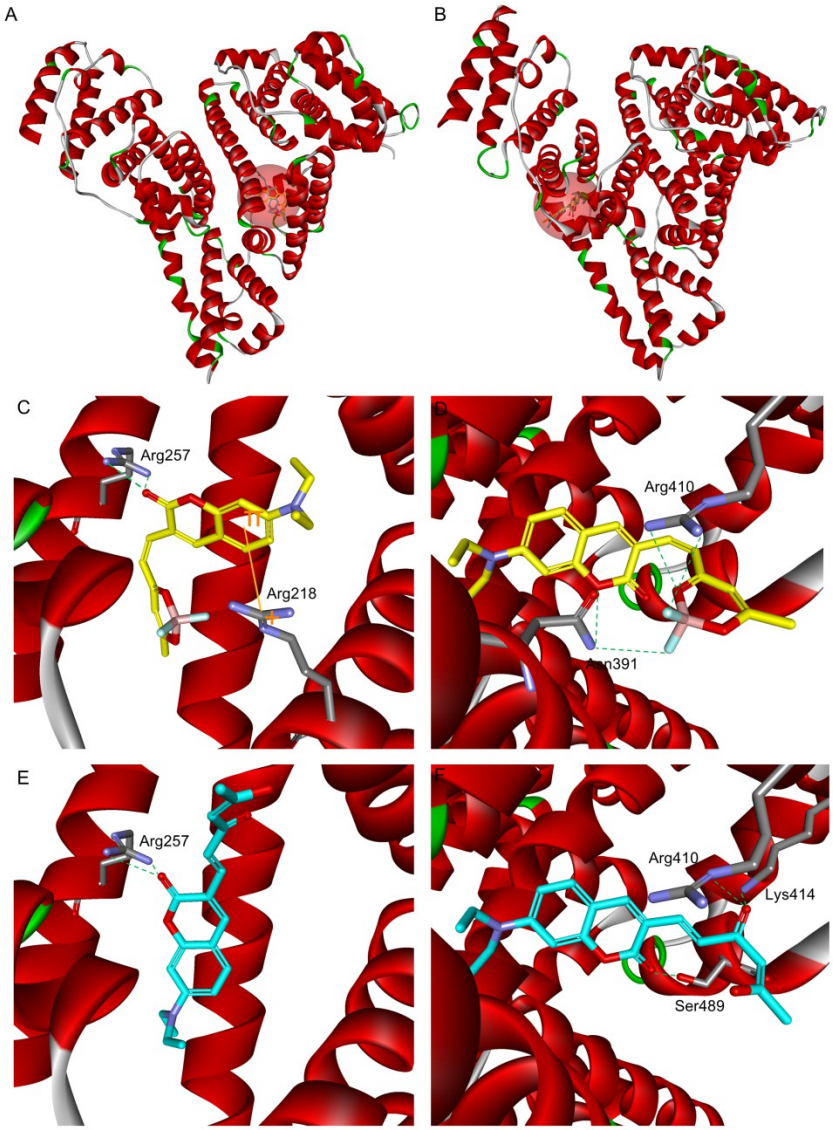


Fig. S8 Predicted binding poses of CBF and RC with HSA. Stereoviews of docking conformations of CBF and RC in the binding site I (A) and site II (B) of HSA. Binding modes of CBF with contacting residues in the binding site I (C) and site II (D) of HSA. Binding modes of RC with contacting residues in the binding site I (E) and site II (F) of HSA. Backbone color code: CBF in yellow; RC in cyan; amino acid residues in grey. H-bonds are displayed as green dashes. Cation-π interaction is displayed as orange solid line.

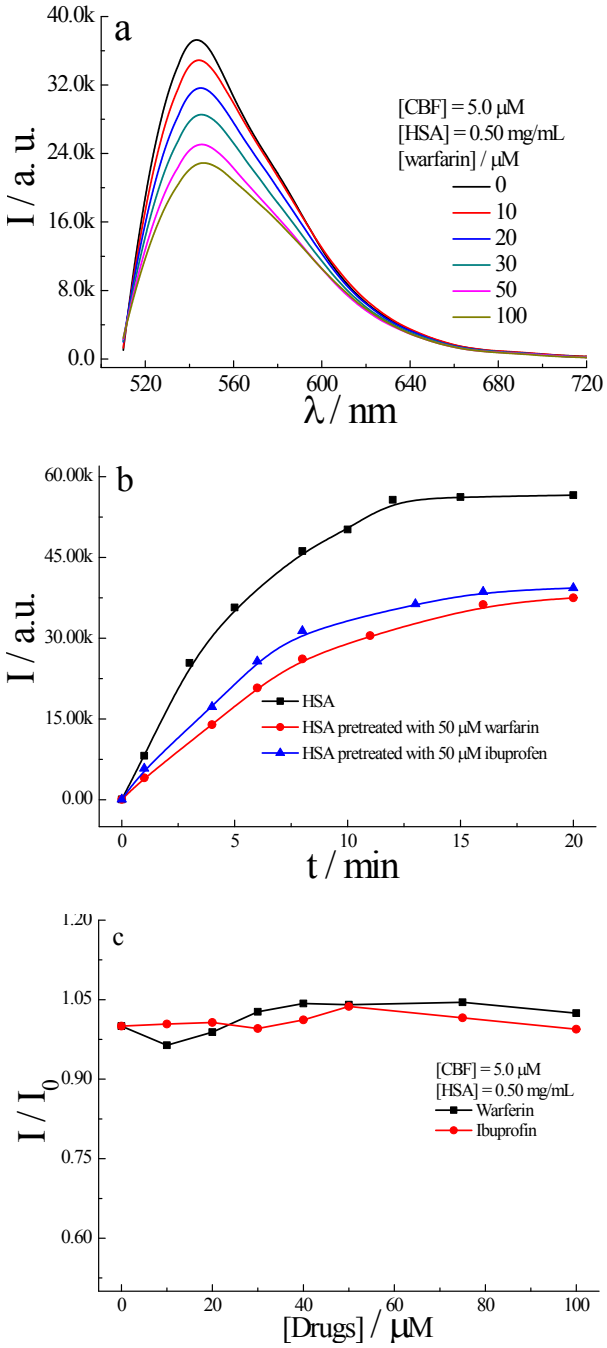


Fig. S9 The fluorescence spectra of CBF in the presence of HSA pretreated with different concentrations of warfarin (a), the fluorescence intensity of CBF in the presence of HSA pretreated with 50 μM of warfarin or ibuprofen (b); effect of drug concentration on the fluorescence intensity ratio I/I_0 of CBF- HSA mixture (c). 20 mM PBS (pH 7.4), [CBF] = 5.0 μM, [HSA] = 0.50 mg/mL, λ_{ex} = 495 nm, λ_{em} = 540 nm.

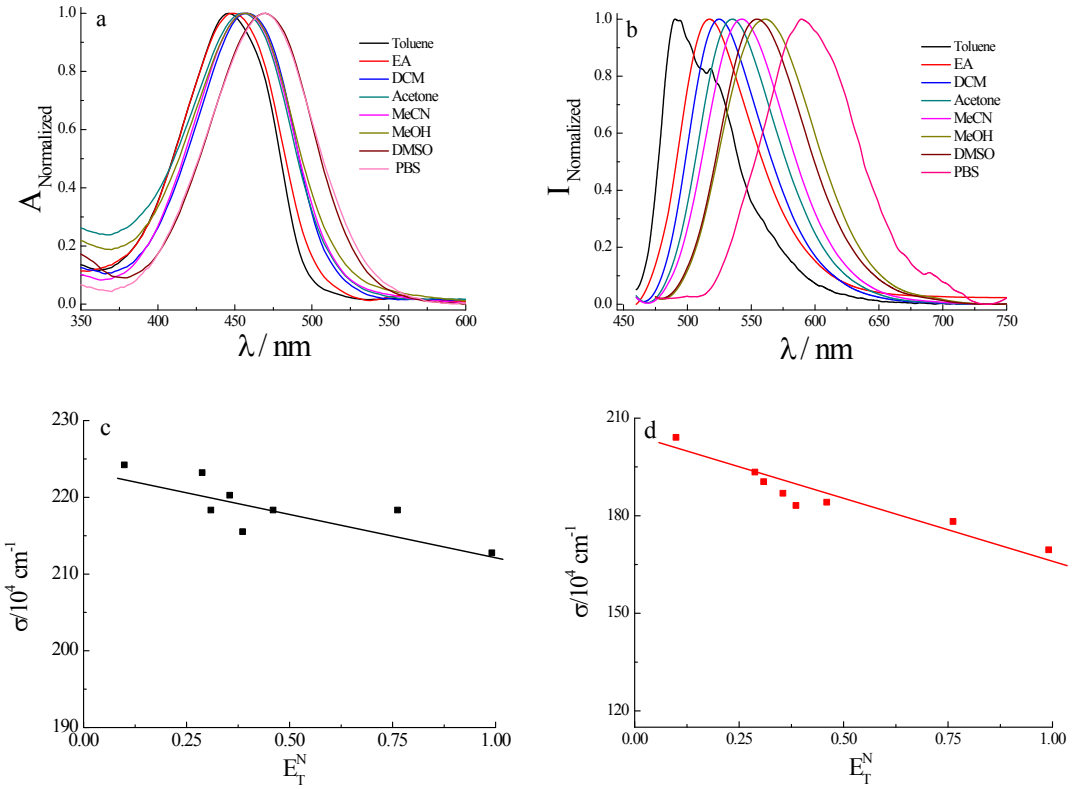


Fig. S10 Normalized absorption (a) and emission (b) spectra of RC in various solvents, and the correlation of absorption (c) and emission (d) wavenumber of RC with solvent polarity. Recorded 5 min after the addition of the probe.

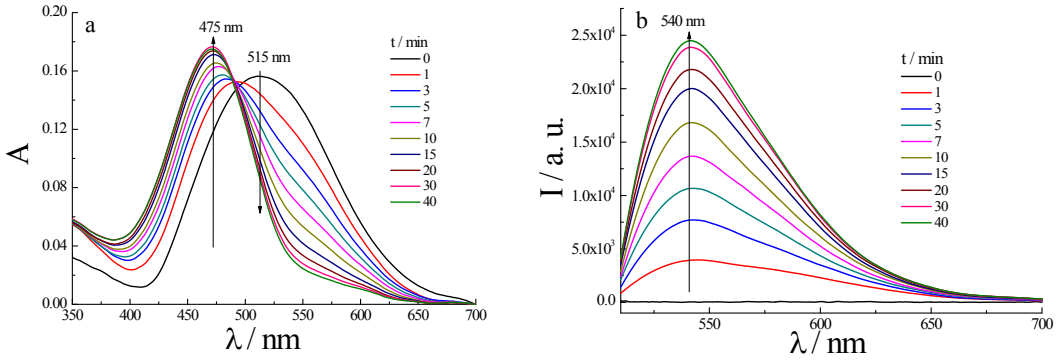


Fig. S11 Time-dependent absorption (a) and emission (b) spectra of CBF mixed with BSA in PBS. [CBF] = 5.0 μM, [BSA] = 1.0 mg/mL, 20 mM PBS (pH 7.4), λ_{ex} = 495 nm.

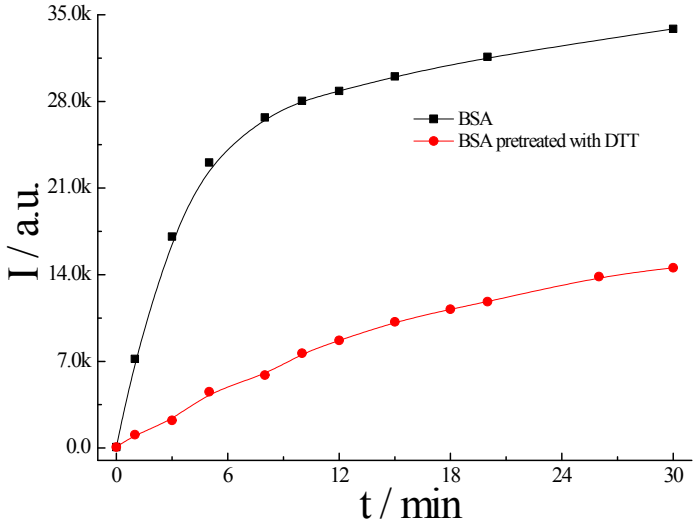


Fig. S12 The fluorescence intensity at 540 nm of CBF as a function of time in the presence of BSA without (black) and with (red) pretreated with DTT in PBS. [CBF] = 5.0 μM, [BSA] = 1.0 mg/mL, 20 mM PBS (pH 7.4), λ_{ex} = 495 nm, λ_{em} = 540 nm.

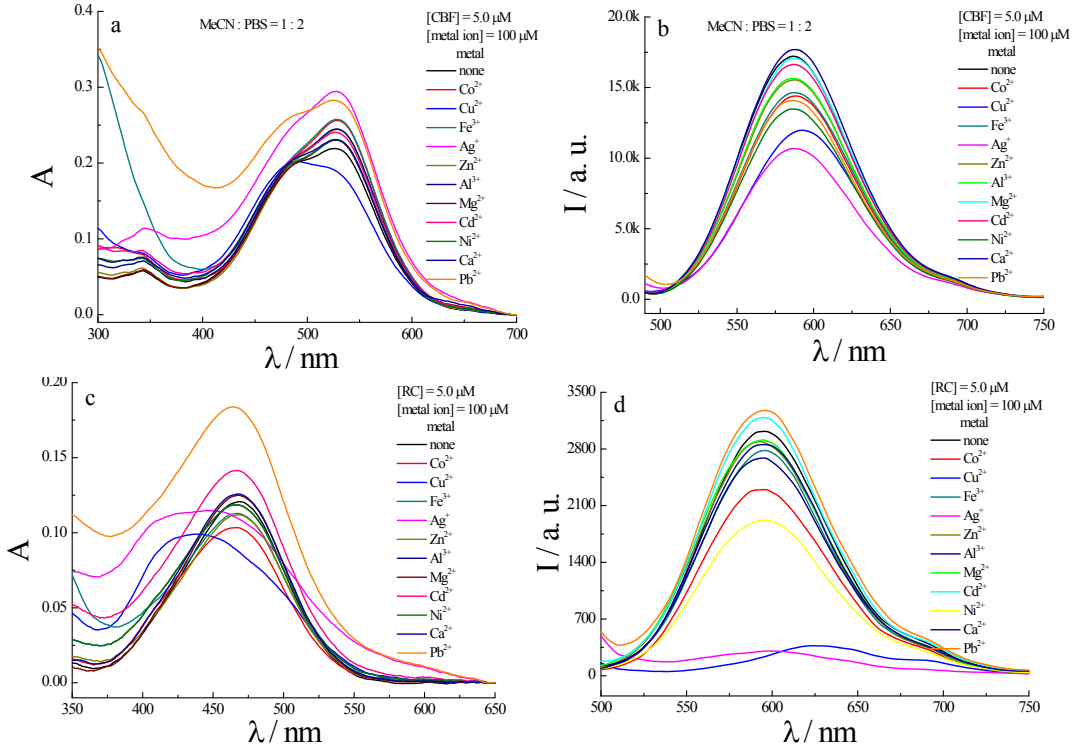


Fig. S13 Effects of metal ions on the absorption (a, c) and emission (b, d) spectra of CBF (a-b) in PBS/MeCN (2:1) and RC (c-d) in PBS. [CBF] = [RC] = 5.0 μM, [metal ion] = 100 μM, [HSA] = 0.50 mg/mL, 20 mM PBS (pH 7.4), λ_{ex} = 490 nm for CBF and 460 nm for RC. Recorded 30 min after each addition.

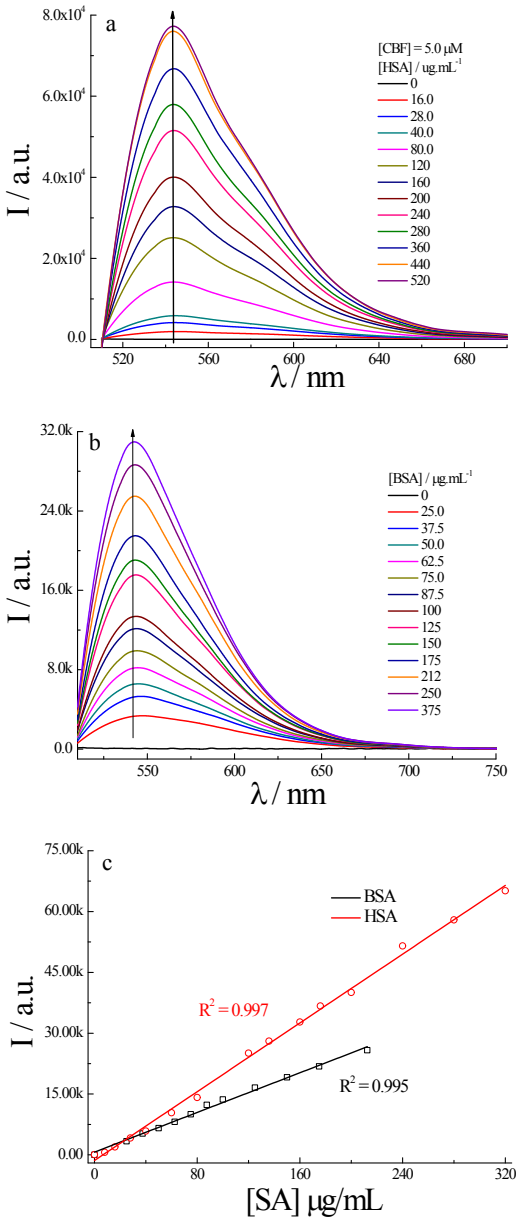


Fig S14 (a-b) Effect of SA concentration on the emission spectrum of CBF in PBS; (c) the fluorescence intensity at 540 nm vs. SA concentration. [CBF] = 5.0 μM, 20 mM PBS (pH 7.4), λ_{ex} = 495 nm, λ_{em} = 540 nm, recorded 30 min after each addition.

Table S1. The photophysical properties of CBF and RC in various solvents.

Compound	Solvent	λ_{ab} / nm	λ_{em} / nm	Stokes shift / nm	Φ_{fl}
CBF	toluene	512	554	42	0.268
	ethylacetate	512	582	70	0.219
	dichloromethane	526	597	71	0.178
	acetone	520	613	93	0.103
	acetonitrile	524	626	104	0.020
	Methanol	516	621	105	0.021
	DMSO	538	635	97	0.046
	PBS [b]	514	/	/	/
RC	toluene	446	490	44	0.735
	ethylacetate	448	517	69	0.621
	dichloromethane	458	525	67	0.510
	acetone	454	535	81	0.484
	acetonitrile	458	543	85	0.387
	Methanol	458	561	103	0.346
	DMSO	470	554	84	0.497
	PBS [b]	470	589	119	0.023

Coumarin 153 (Φ_f = 0.380 in ethanol) was used as the reference; [b] containing 1% DMSO.