Electronic Supplementary Material (ESI) for ChemComm.

An NIR-emitting cyanine dye with pyridinium groups: The impact of regio-bond connection on the photophysical properties

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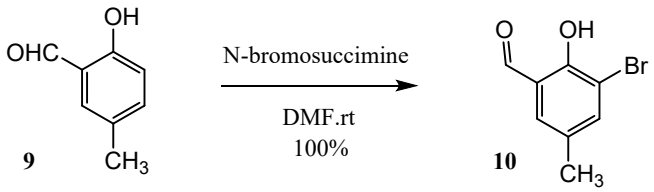
General Considerations: All starting materials and the essential solvents were purchased from Sigma-Aldrich, TGI, Ark Pharma, Fischer Scientific, Alfa-Asaer and Across Organics and directly used without further purification. pH buffers were provided by Mettler Toledo. All deuterated solvents were purchased from Cambridge Isotopes and used as received. All NMR data were recorded on Varian 300 and 500 MHz instruments with all spectra referenced to deuterated solvents. Mass spectra were obtained from HP1100LC/MSD mass spectrometry. HRMS data were performed on an ESI-TOF MS system (Waters, Milford, MA).

Synthesis of 3-bromo-2-hydroxy-5-methylbenzaldehyde (**10**)



1.36 g (10 mmol) 2-hydroxy-5-methylbenzaldehyde (**9**) and 2.29 g (13 mmol) N-bromosuccimine was to 15 ml dimethylformamide (DMF)in round bottom flask. The flask was capped, and the resulting mixture was stirred at room temperature for 5h. Aqueous saturated citric acid solution was added followed by adding ethyl acetate to extract for three times. The combined ethyl acetate solution was washed with saturated aqueous NaCl solution three times. After removing the solvent on a rotary evaporator, the solid residue was collected to give orange-yellow solid product **10** in almost 100 % yield.

^{1}H-NMR (300 MHz, DMSO-d_6) δ= 11.00 (s, 1H), 10.00 (s, 1H), 7.72 (s, 1H), 7.54 (s, 1H), 2.26 (s, 3H).

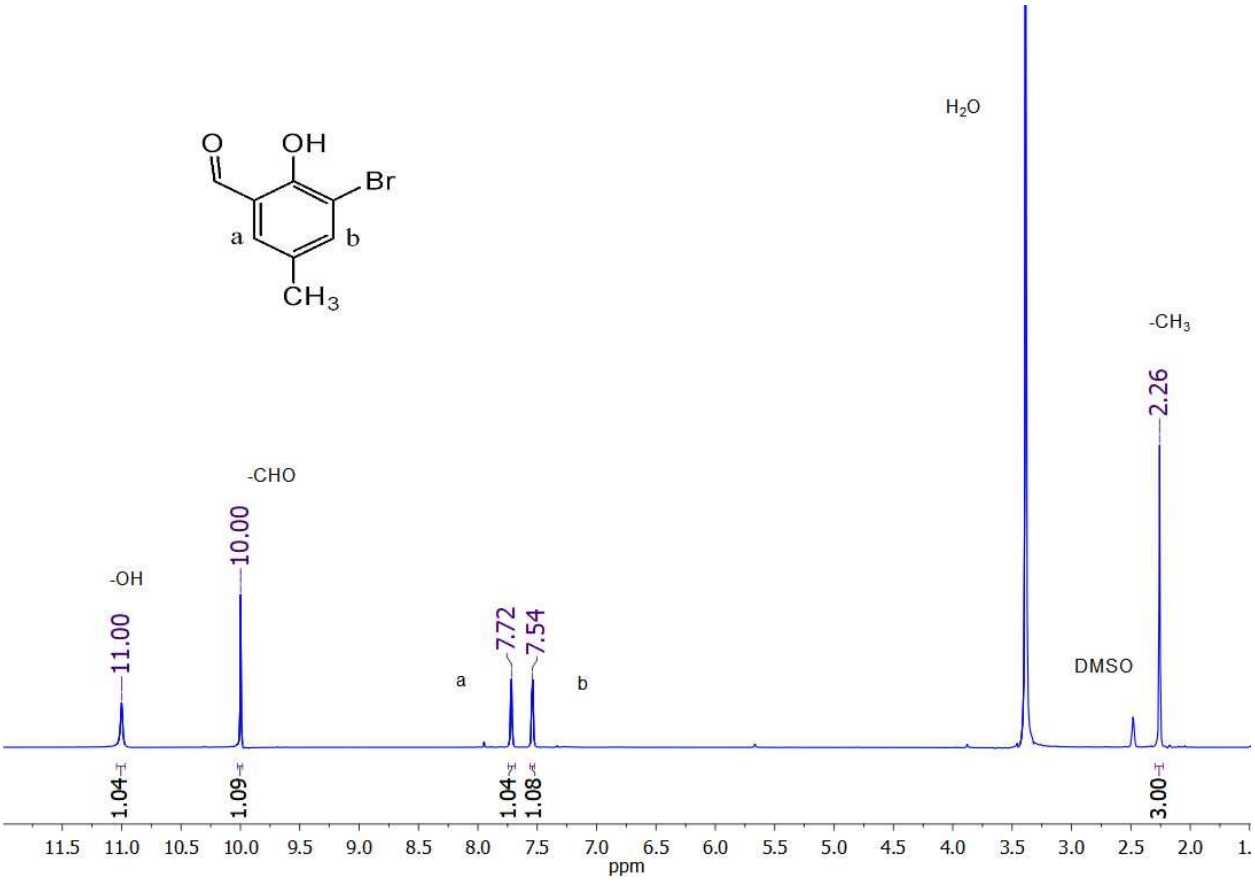
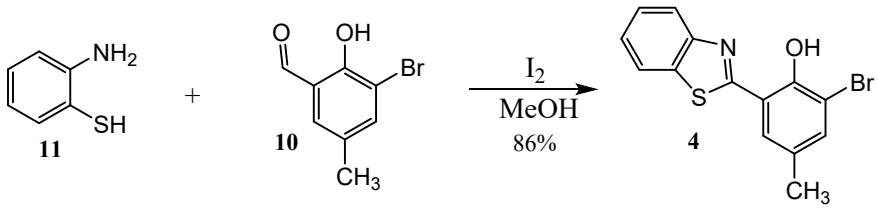


Fig. s1 ^{1}H-NMR spectra of compound **10**.

Synthesis of 2-(benzo[d]thiazol-2-yl)-6-bromo-4-methylphenol (**4**)



0.45 g (1.8 mmol) 2-aminothiophenol (**6**) and 0.31 g (2.4 mmol) compound **5** were added to 15 ml methanol in round bottom flask with I_2 as catalyst. The resulting mixture was stirred in 40℃ overnight. The white product was collected by vacuum filtration and washed with methanol. After drying under vacuum, compound **4** was obtained in 85% yield.

^{1}H-NMR (300 MHz, DMSO-d_6) δ= 12.62 (s, 1H), 8.18 (d, 1H), 8.08 (d, 1H), 7.75 (s, 1H), 7.61 (t, 2H), 7.50 (t, 1H), 2.32 (s, 1H).

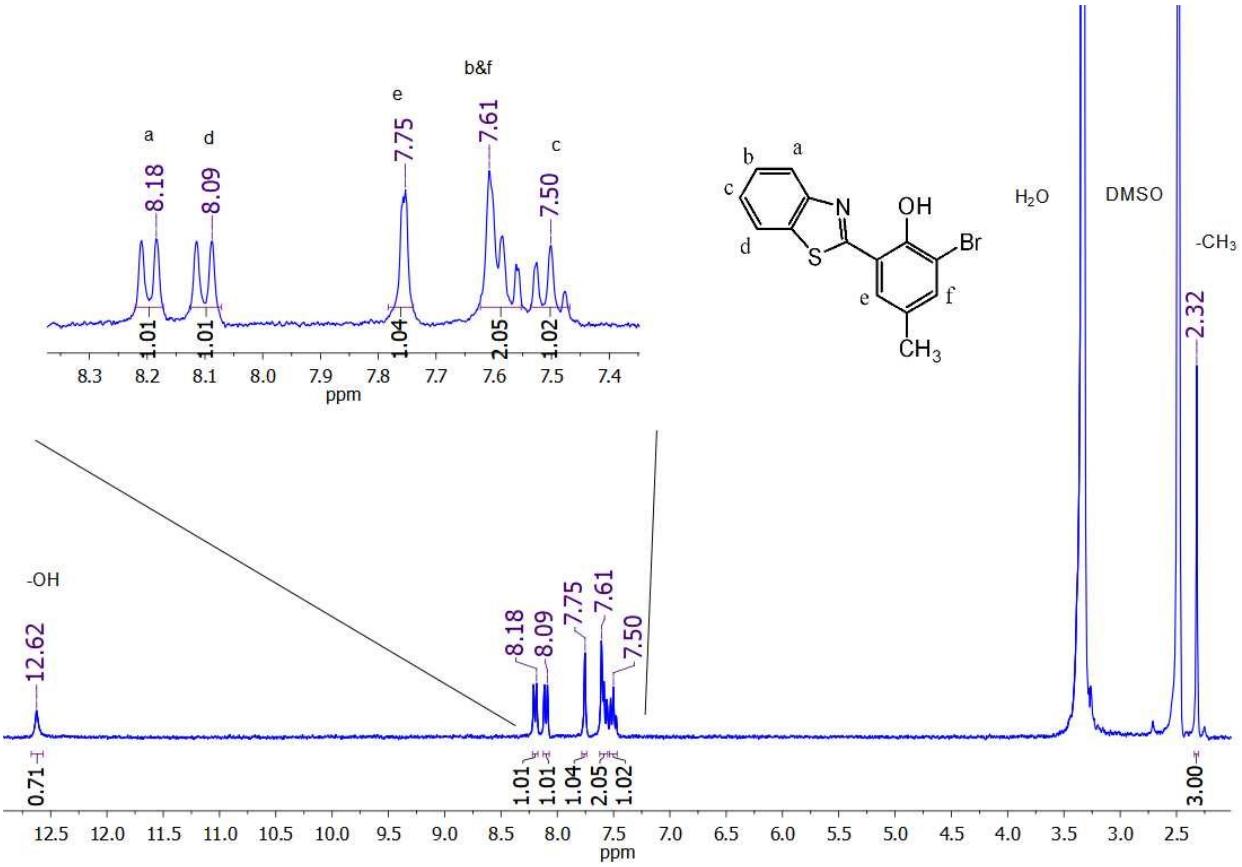
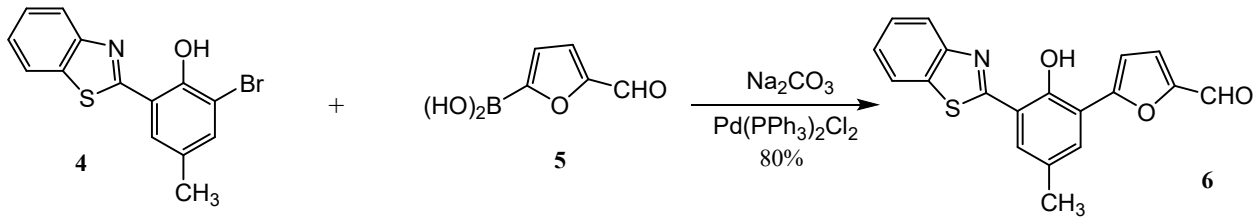


Fig. s2 ^{1}H-NMR spectra of compound **4**.

Synthesis of 5-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl) furan-2-carbaldehyde (**6**)



To the DMF solution (12 mL) of compound **4** (0.32 g, 1 mmol) was added $Pd(PPh_3)_2Cl_2$ (12 mg) as Suzuki coupling catalyst in two way round bottom flask and fill with argon. 3 ml DMF solution of 5-Formylfuran-2-boronic acid (**5**) (0.16 g, 1.1 mmol) and 5 ml of aqueous Na_2CO_3 were added to the flask by syringe under argon protection. The resulting mixture was stirred at 80℃ overnight. After vacuum filtration, the filtrate was extracted by 30 ml ethyl acetate three times. The combined solution was washed by saturated aqueous NaCl solution and evaporated solvent by a rotary evaporator to give light yellow solid product **6** in 80% yield.

^{1}H-NMR (500 MHz, DMSO-d_6) δ= 12.63 (s, 1H), 9.63 (s, 1H), 8.06-8.26 (m, 3H), 7.67-7.84 (m, 2H), 7.61 (t, 2H), 7.50 (t, 1H), 2.32 (s, 3H).

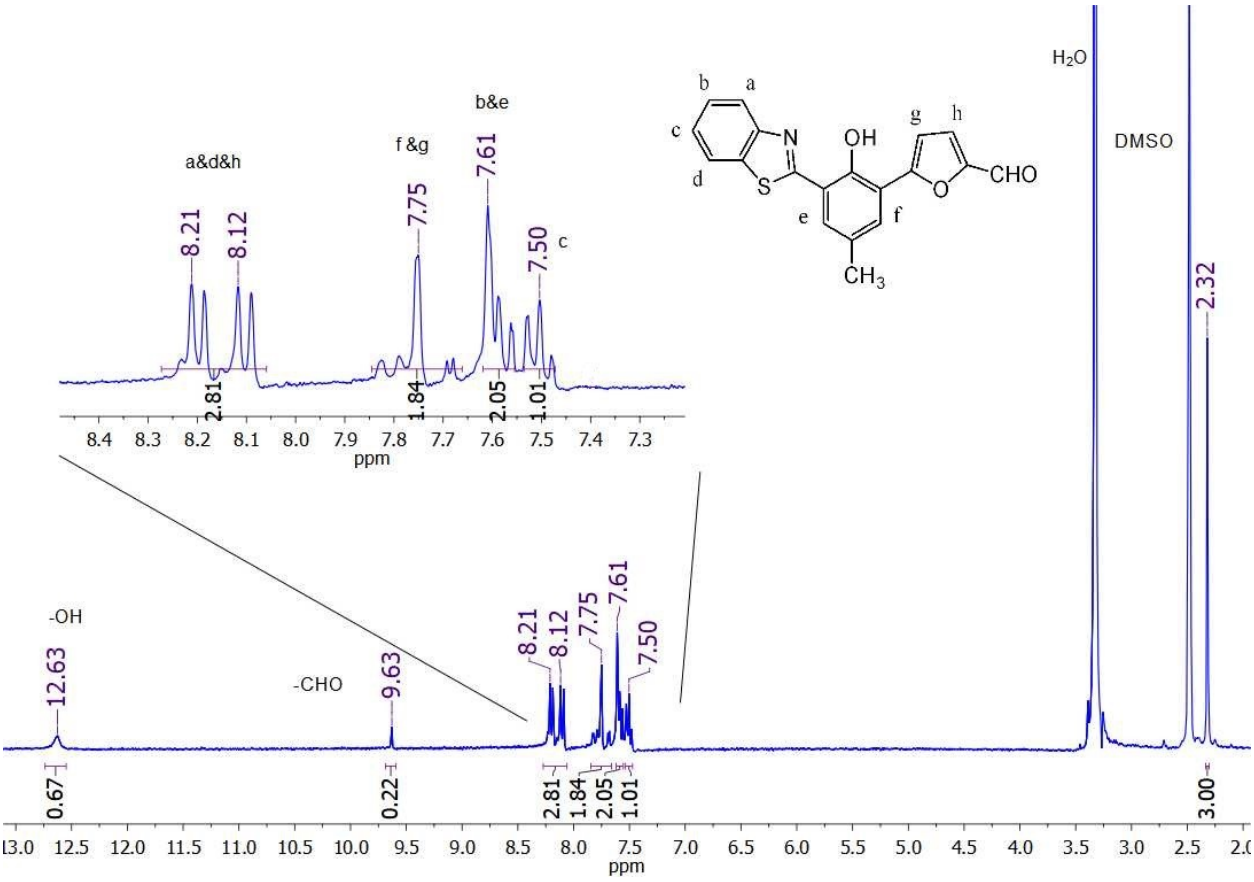
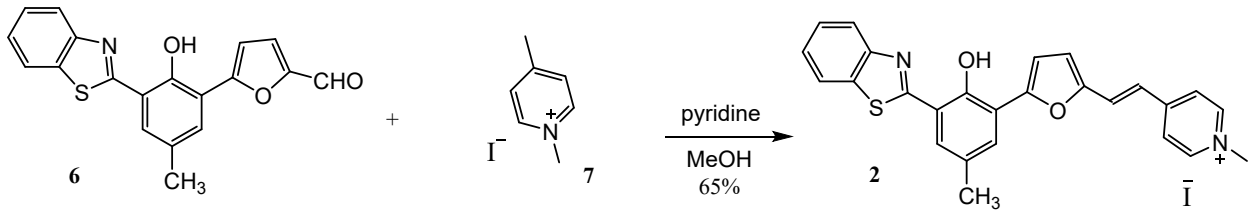


Fig. s3 ^{1}H-NMR spectra of compound **6**.

Synthesis of (E)-4-(2-(5-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl) furan-2-yl) vinyl)-1-methylpyridin-1-ium iodide (**2**)



To the methanol solution (3 mL) of compound **6** (0.060 g, 0.18 mmol) was added compound **7** (0.058 mg, 0.25 mmol) and pyridine (0.5mL) in round bottom flask. The resulting mixture was heated to reflux overnight. After collecting by filtration, the crude product was washed with hot water and ethyl acetate several times respectively and dried under vacuum to give dark red solid product **2** in 65% yield.

^{1}H-NMR (500 MHz, DMSO-d_6) δ= 13.31 (s, 1H), 8.77 (d, 2H), 8.17 (d, 3H), 8.10 (d, 1H), 7.87 (d, 2H), 7.65 (s, 1H), 7.59 (t, 1H), 7.51 (t, 1H), 7.33 (d, 1H), 7.30 (d, 1H), 7.07 (d, 2H), 4.20 (s, 3H), 2.43 (s, 3H).

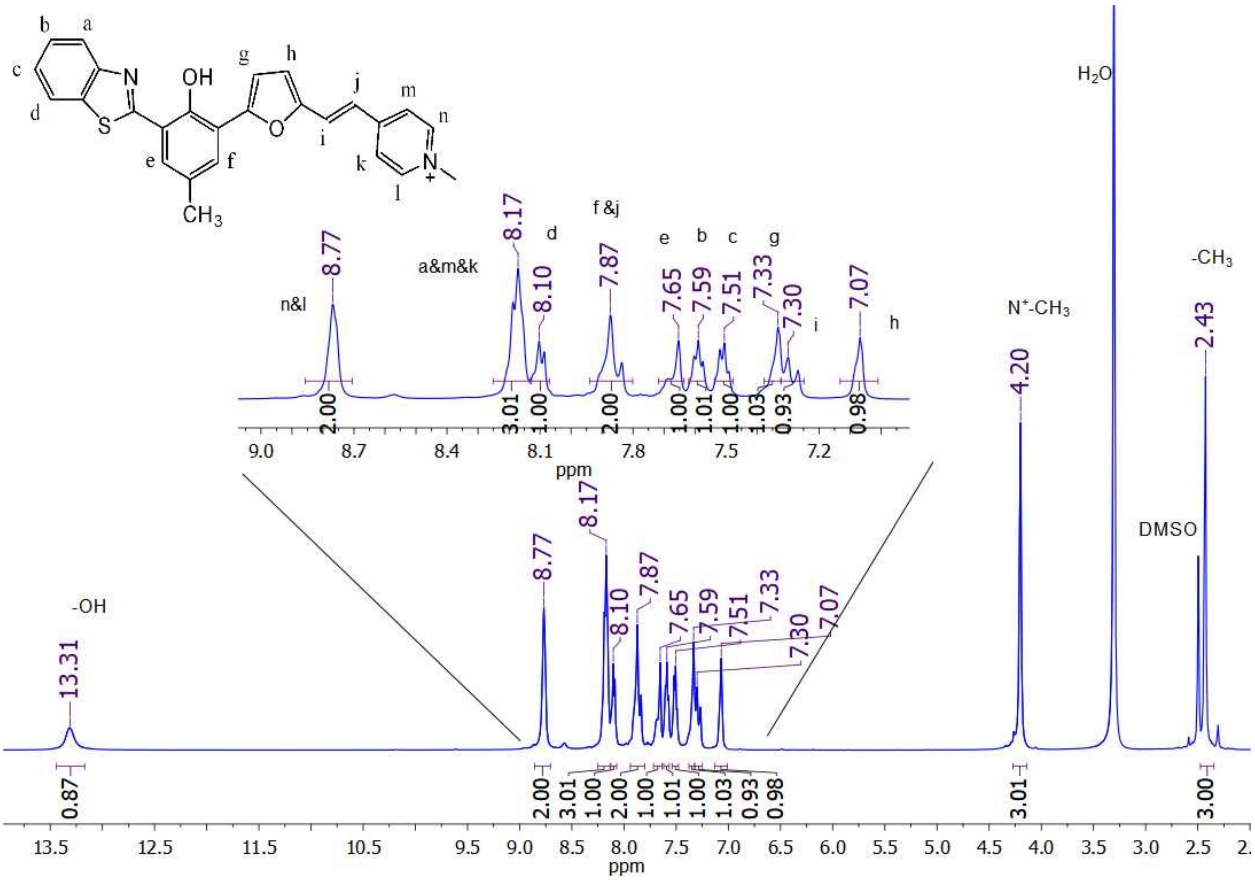


Fig. s4 ^{1}H-NMR spectra of compound **2**.

^{13}C- NMR (126 MHz, DMSO-d_6) δ= 169.30, 152.77, 152.57, 152.44, 151.28, 150.60, 150.05, 145.18, 132.71, 129.80, 129.09, 127.63, 127.33, 126.49, 123.48, 122.84, 122.36, 120.81, 119.21, 118.42, 117.13, 114.27, 47.19, 20.63.

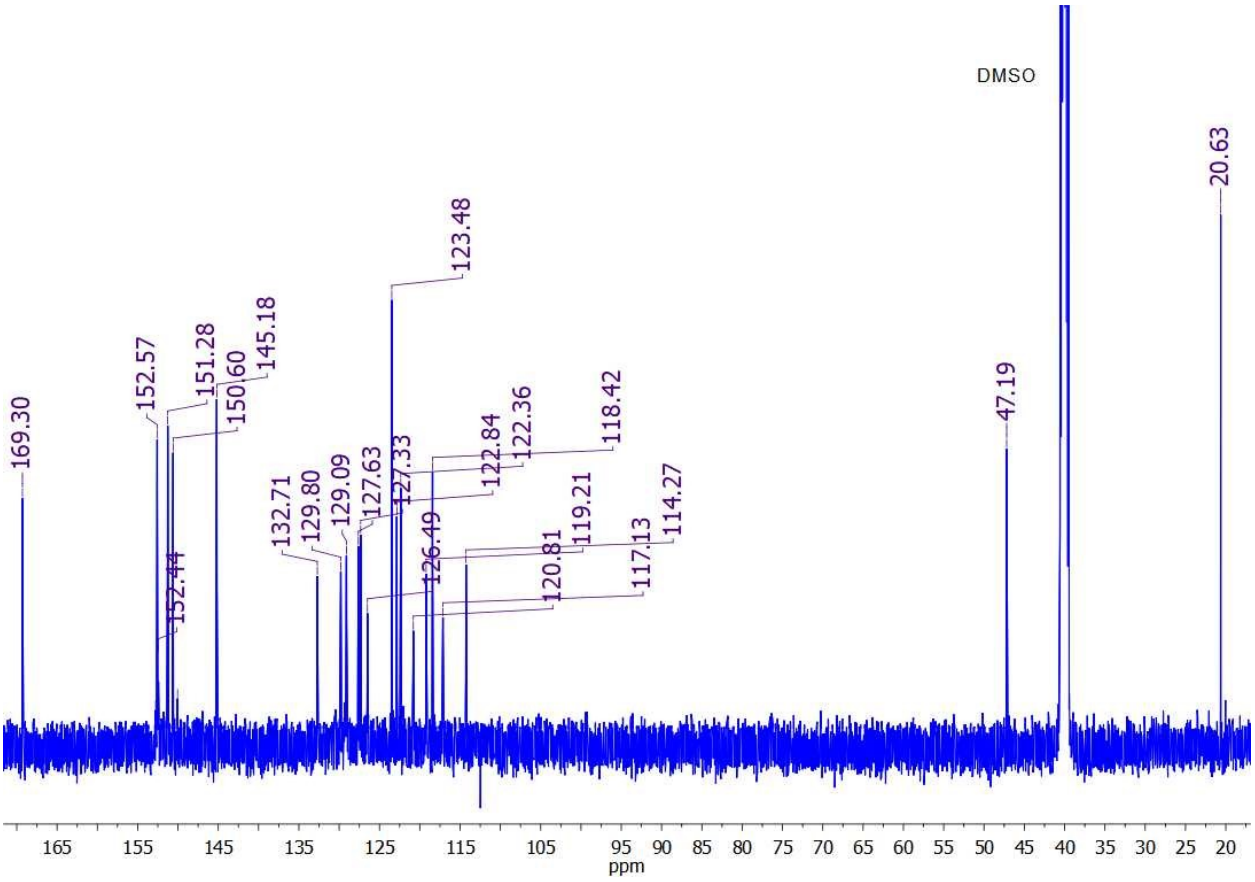


Fig. s5 ^{13}C-NMR spectra of compound **2**.

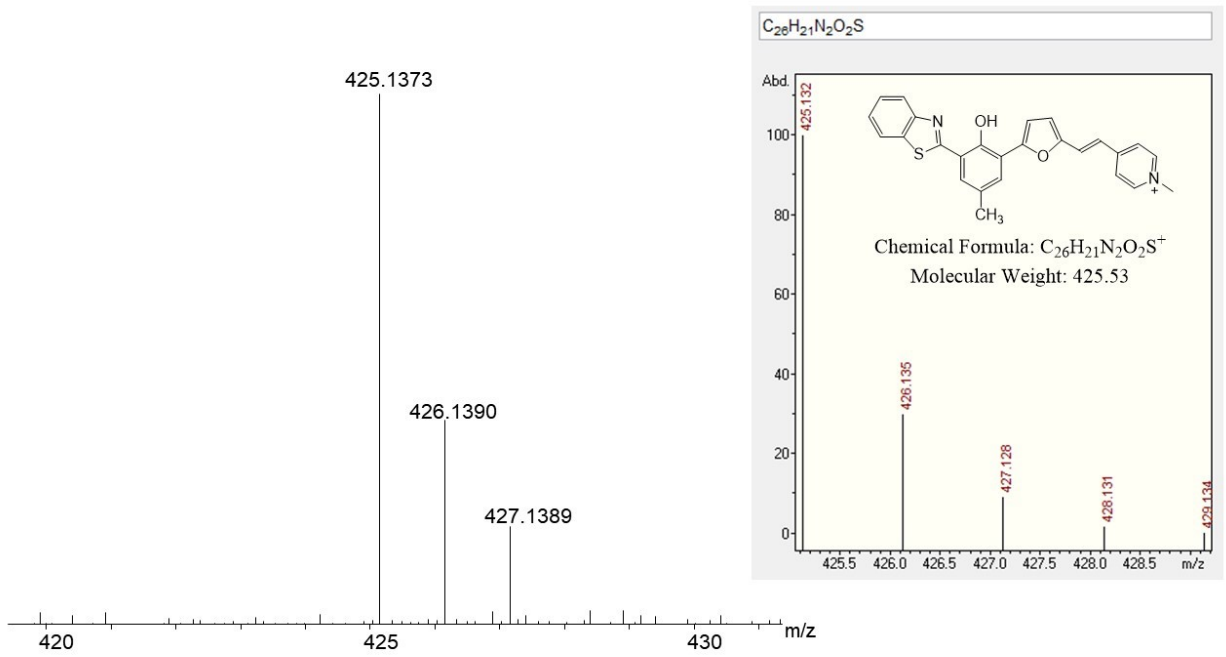
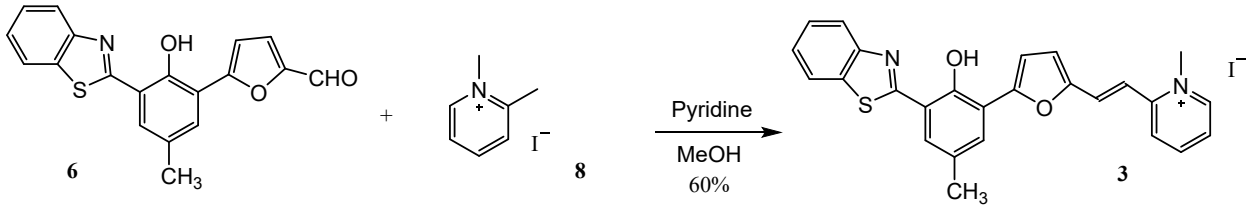


Fig. s6 ESI- MS spectra of compound **2**.

Synthesis of (E)-2-(2-(5-(3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylphenyl) furan-2-yl) vinyl)-1-methylpyridin-1-ium iodide (**3**)



To the methanol solution (3 mL) of compound **9** (0.060 g, 0.18 mmol) was added compound **11** (0.058 mg, 0.25 mmol) and pyridine (0.5mL) in round bottom flask. The resulting mixture was heated to reflux for 24h. After collecting by filtration, the crude product was washed with hot water and ethyl acetate several times respectively and dried under vacuum to give dark red solid product **3** in 60% yield.

^{1}H-NMR (500 MHz, DMSO-d_6) δ= 13.32 (s, 1H), 8.85 (d, 1H), 8.46 (d, 1H), 8.42 (t, 1H), 8.18 (d, 1H), 8.10 (d, 1H), 7.96 (s, 1H), 7.87 (d, 1H), 7.72 (s, 1H), 7.59 (t, 1H), 7.50 (t, 1H), 7.41 (d, 1H), 7.36 (d, 1H), 7.22 (d, 1H), 4.39 (s, 3H), 2.43 (s, 3H).

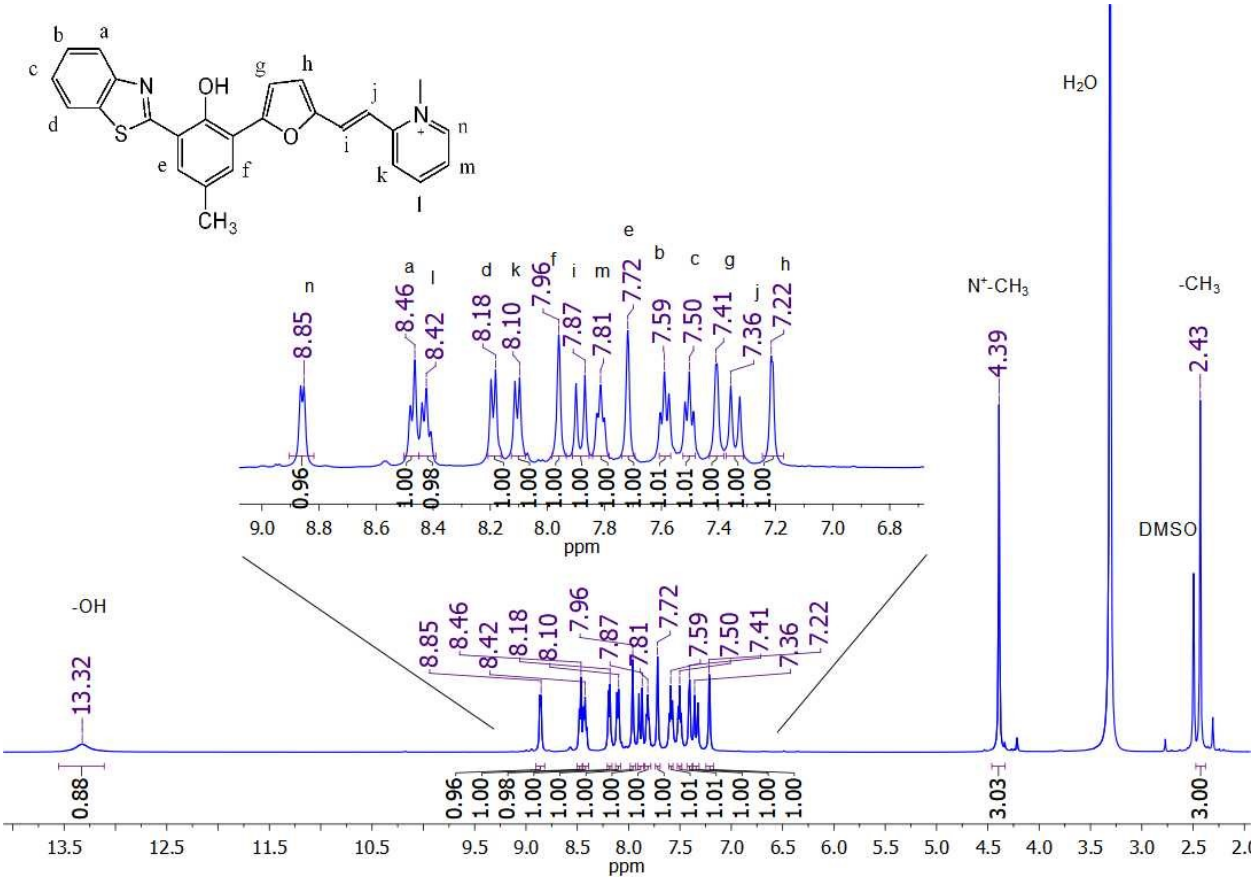


Fig. s7 ^{1}H-NMR spectra of compound **3**.

^{13}C- NMR (126 MHz, DMSO-d_6) δ= 169.11, 153.30, 152.46, 151.35, 150.25, 146.33, 144.23, 136.56, 132.93, 132.84, 130.03, 129.30, 127.53, 126.33, 124.84, 124.74, 122.81, 122.29, 120.23, 118.34, 117.42, 114.22, 114.13, 114.02, 46.37, 20.63.

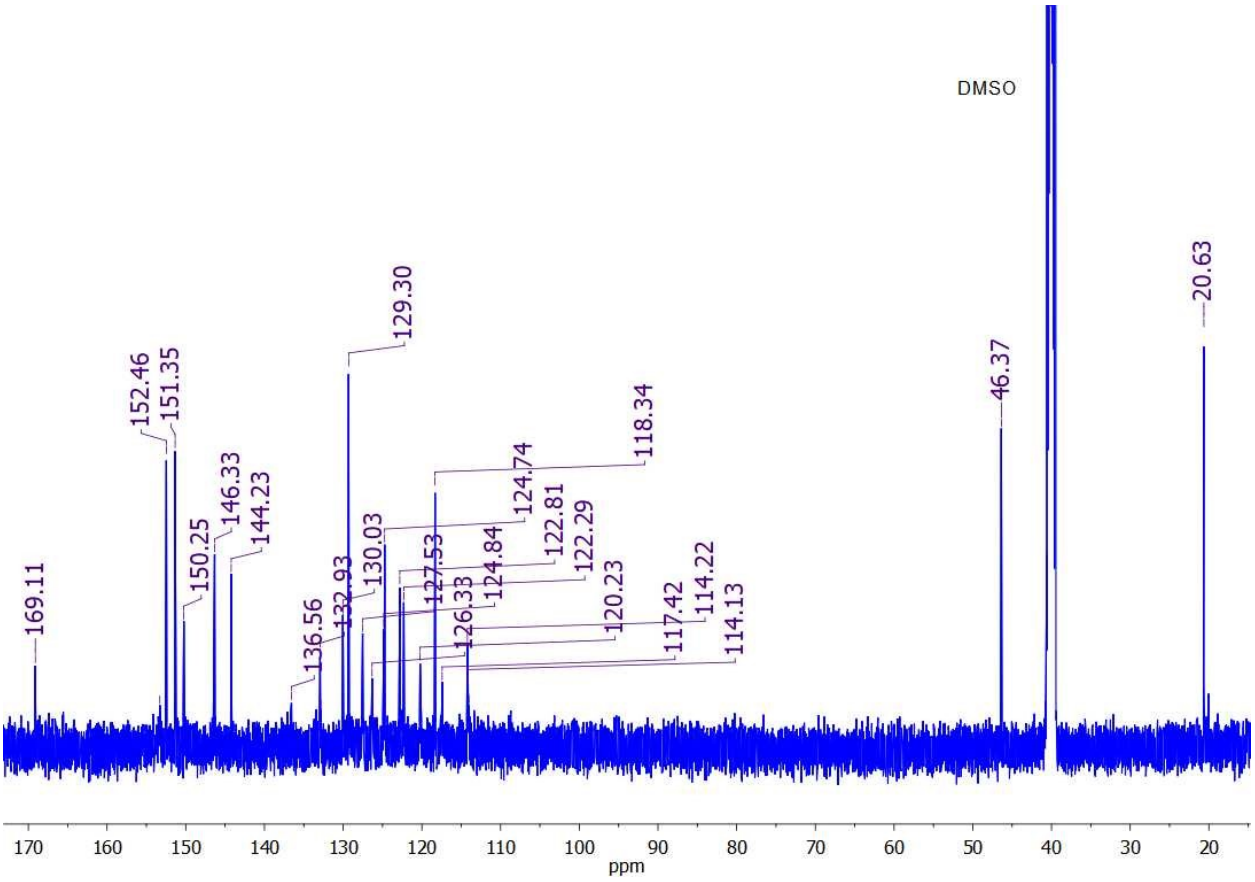


Fig. s8 ^{13}C-NMR spectra of compound **3**.

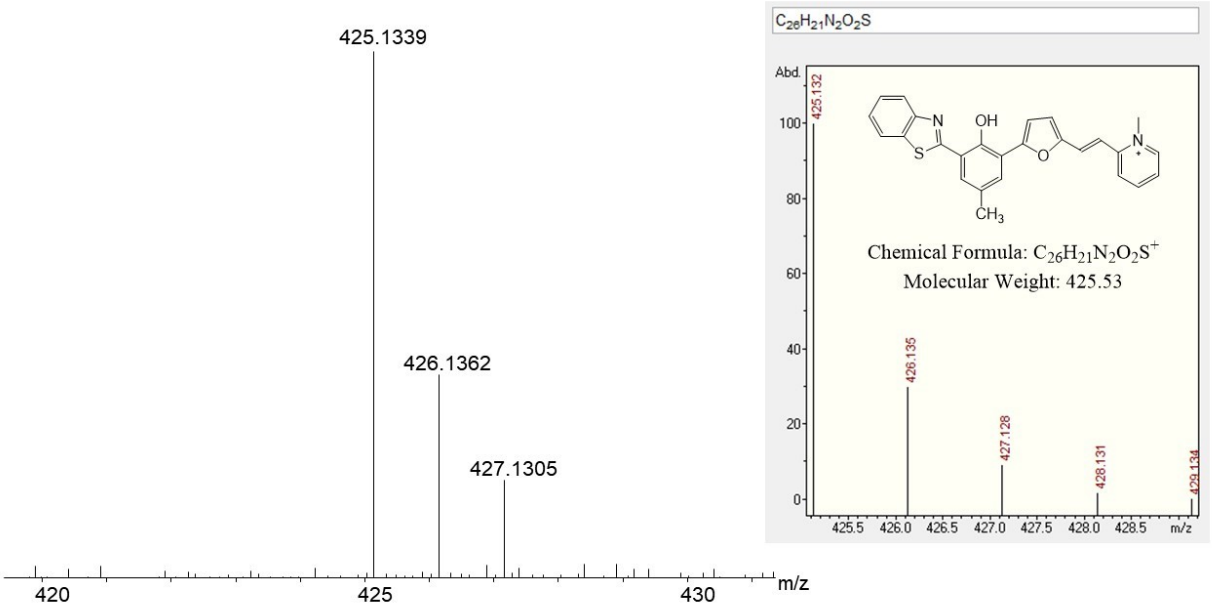


Fig. s9 ESI- MS spectra of compound **3**.

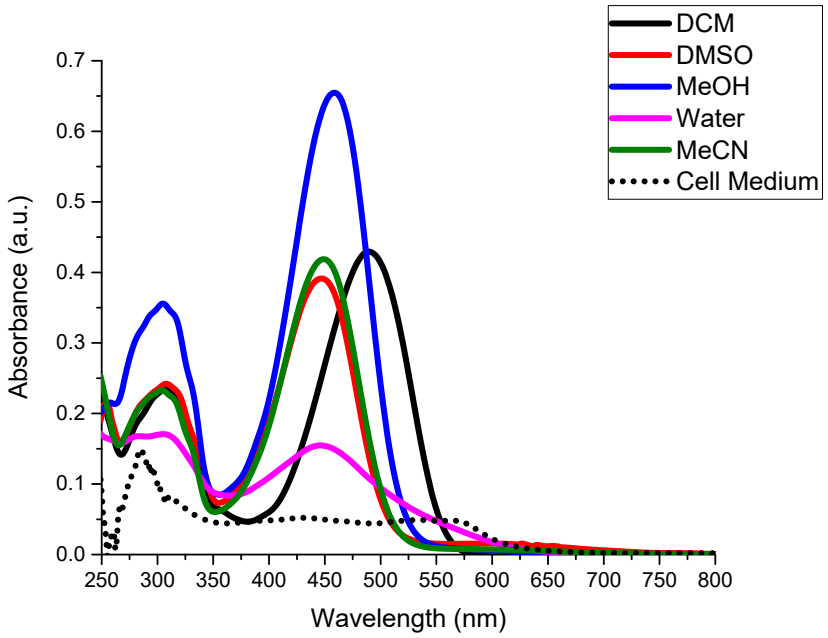


Fig. s10 UV-absorbance spectra of compound **2** (10 µM) in different solvents.

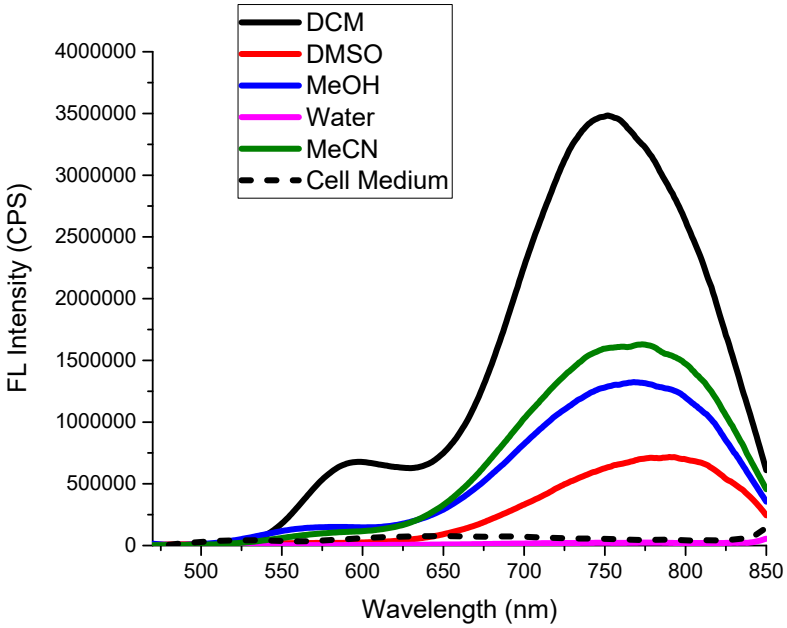


Fig. s11 Fluorescence spectra of compound **2** (10 µM) in different solvents.

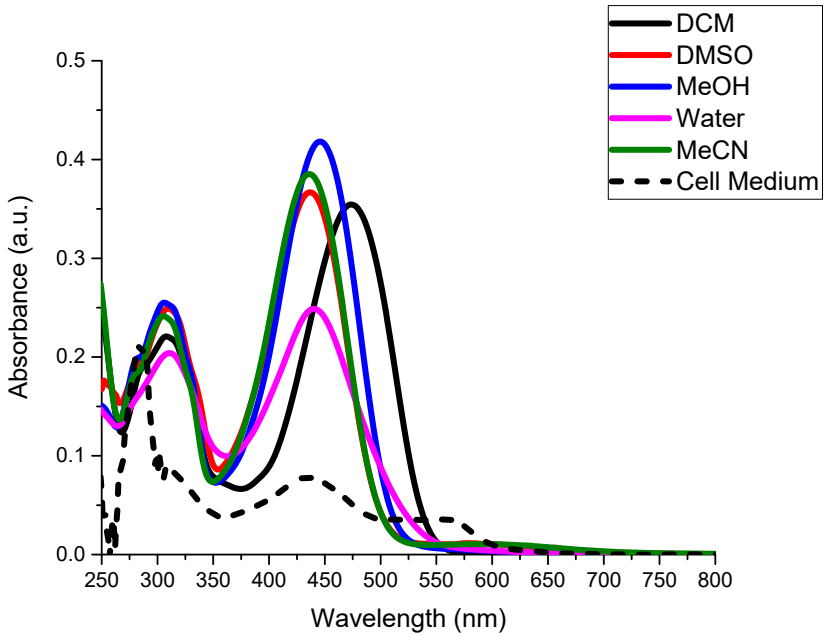


Fig. s12 UV-absorbance spectra of compound **3** (10 µM) in different solvents.

The absorption spectrum of **3** in cell medium revealed an additional peak at ~565 nm, which can be attributed to the deprotonated species since the pH of cell medium is 7.4.

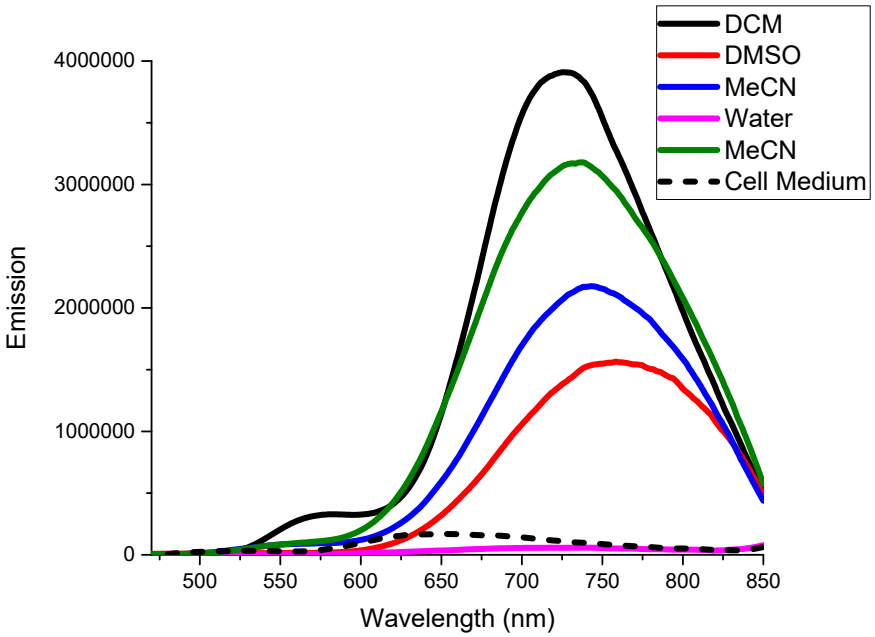


Fig. s13 Fluorescence spectra of compound **3** (10 µM) in different solvents.

Calculation of quantum yield:

Rhodamine 6G ($\Phi_{ref} \approx 0.94$) was used as a standard of reference for compound 5. Quantum yield of probes was calculated using an equation shown below, where 'A' is absorbance at the excitation wavelength, 'I' is an integrated area of emission, 'ϕ_{ref}' is a quantum yield of reference compound and 'η' is the refractive index of solvent used.

$$\phi_{Sample} = \phi_{ref} * (I_{Sample}/ I_{ref}) * (A_{ref}/ A_{Sample}) * (\eta_{Sample}/ \eta_{ref})^2$$

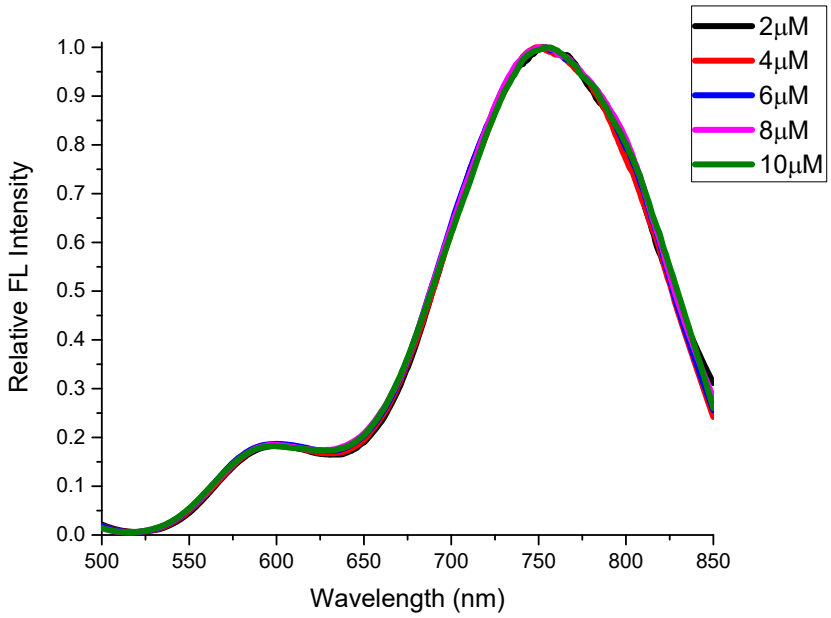


Fig. s14 Normalized fluorescence spectra of **2** with increased concentration in CH_2Cl_2 (λ_{ex}=490 nm). Normalized FL Intensity = The original FL Intensity/ Max. FL Intensity (at ~750 nm).

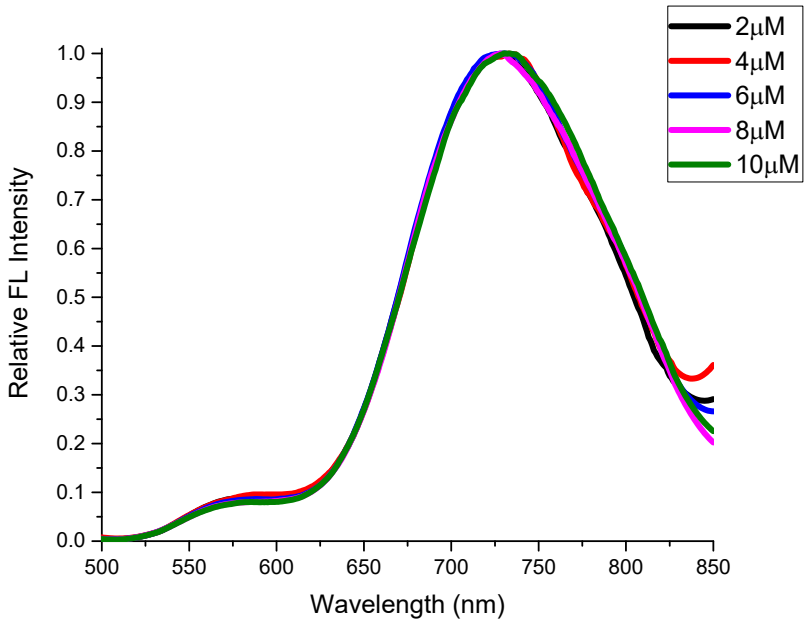


Fig. s15 Normalized fluorescence spectra of **3** with increased concentration in CH_2Cl_2 ($(\lambda_{ex}$=474 nm). Normalized FL Intensity= The original FL Intensity/ Max. FL Intensity (at ~750 nm).

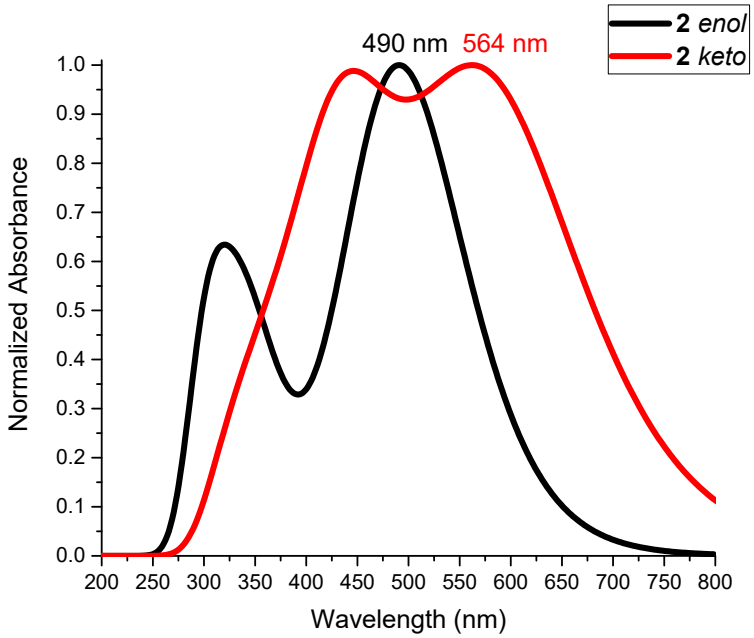


Fig. s16 The calculated UV-Vis spectra of enol and keto tautomer of **2** at the B3LYP/6-31G (d, p) level in DCM. The spectra were generated by using TD-SCF method, after the molecular geometry was optimized at the B3LYP/6-31G (d, p) level.

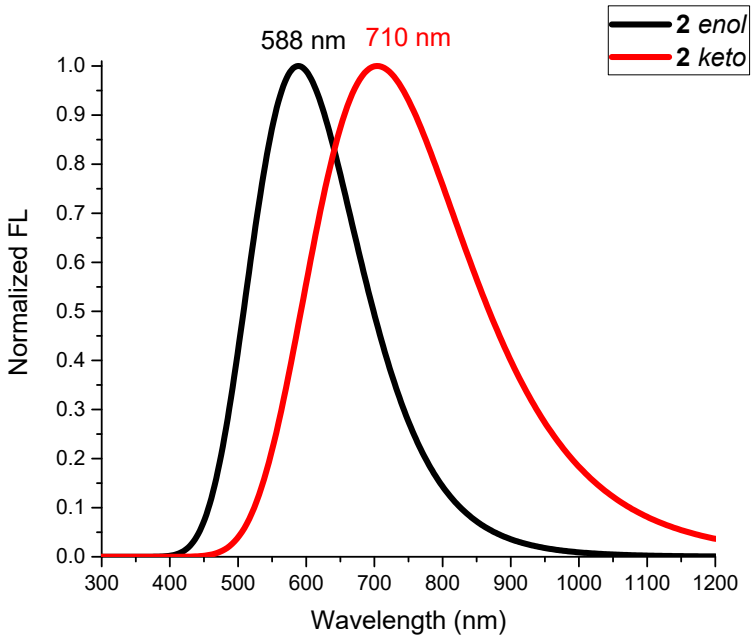


Fig. s17 The calculated fluorescence spectra of enol and keto tautomer of **2** at the B3LYP/6-31G (d, p) level in DCM. The spectra were generated by using TD-SCF method, after the molecular geometry was optimized at the B3LYP/6-31G (d, p) level.

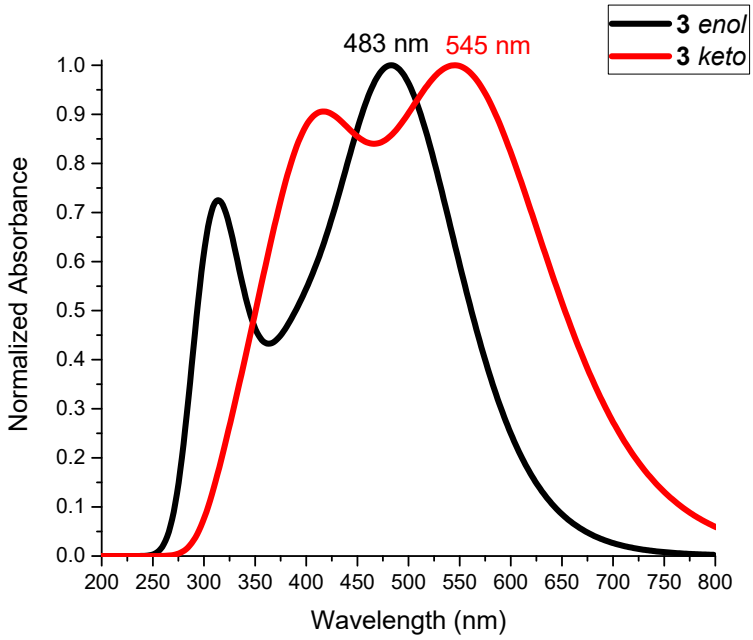


Fig. s18 The calculated UV-Vis spectra of enol and keto tautomer of **3** at the B3LYP/6-31G (d, p) level in DCM. The spectra were generated by using TD-SCF method, after the molecular geometry was optimized at the B3LYP/6-31G (d, p) level.

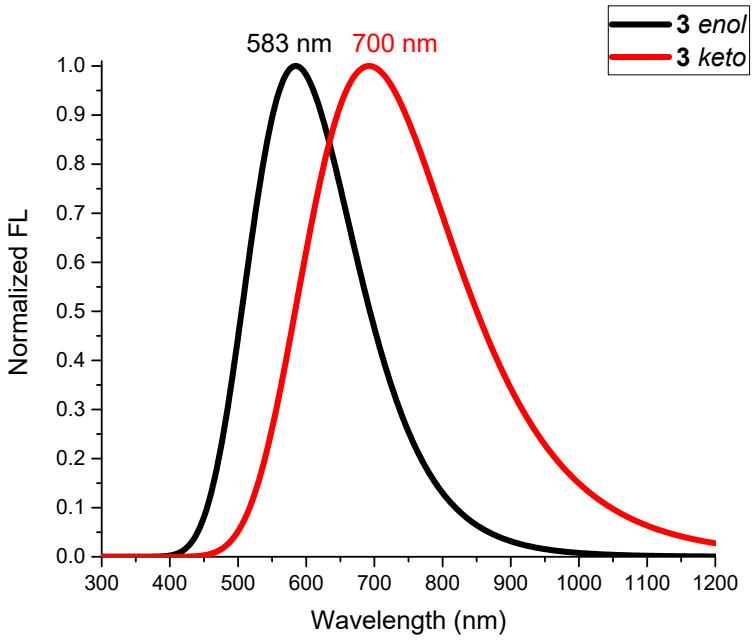


Fig. s19 The calculated fluorescence spectra of enol and keto tautomer of **3** at the B3LYP/6-31G (d, p) level in DCM. The spectra were generated by using TD-SCF method, after the molecular geometry was optimized at the B3LYP/6-31G (d, p) level.

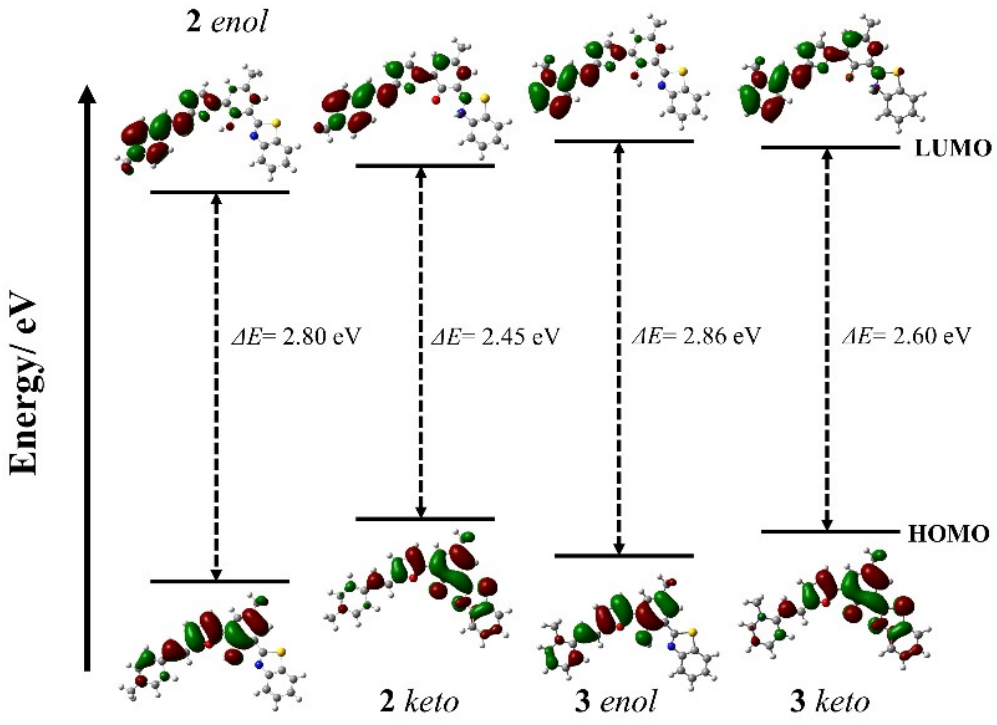


Fig. s20 HOMO- LUMO and energy gap of enol and keto tautomer of **2** and **3.**

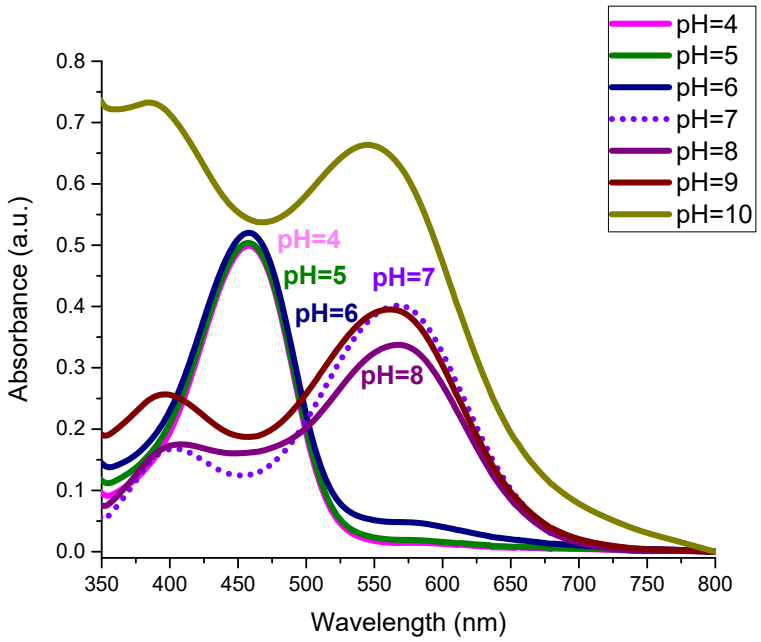


Fig. s21 UV-Vis spectra of **2** (10 μM) in pH buffers.

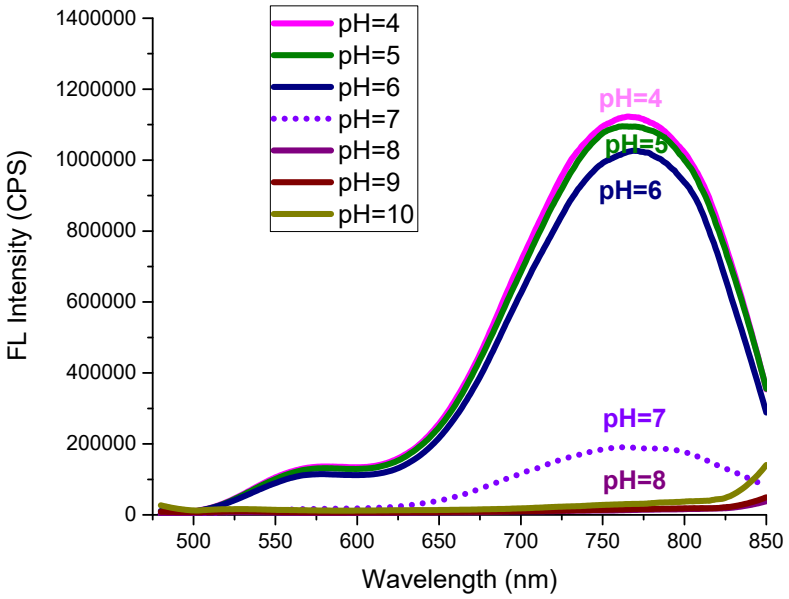


Fig. s22 Fluorescent spectra of **2** (10 μM) in pH buffers (λ_{ex}=460 nm).

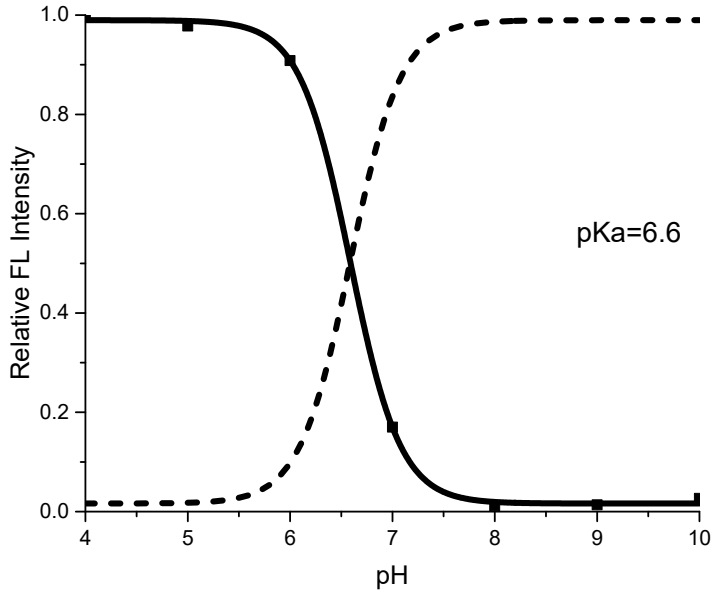


Fig. s23 Response curve of **2** to pH and pKa calculation.

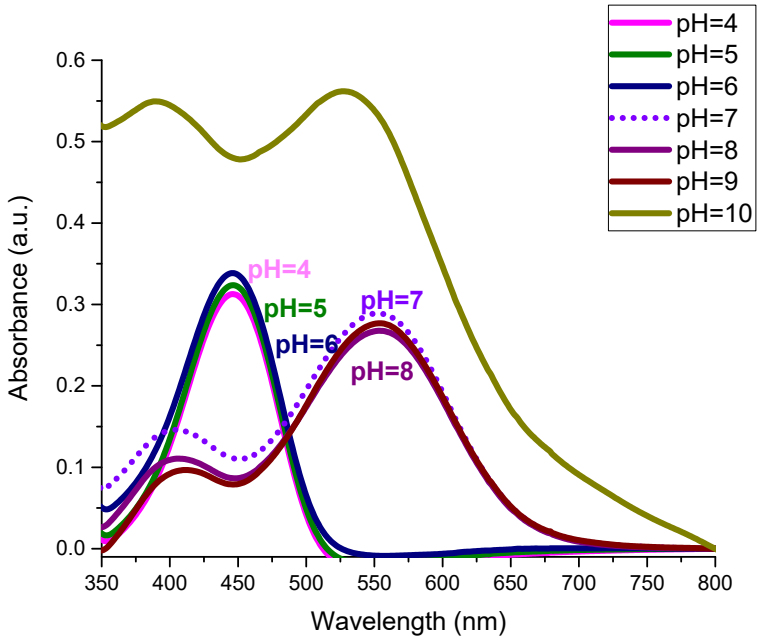


Fig. s24 UV-Vis spectra of **3** (10 µM) pH buffers.

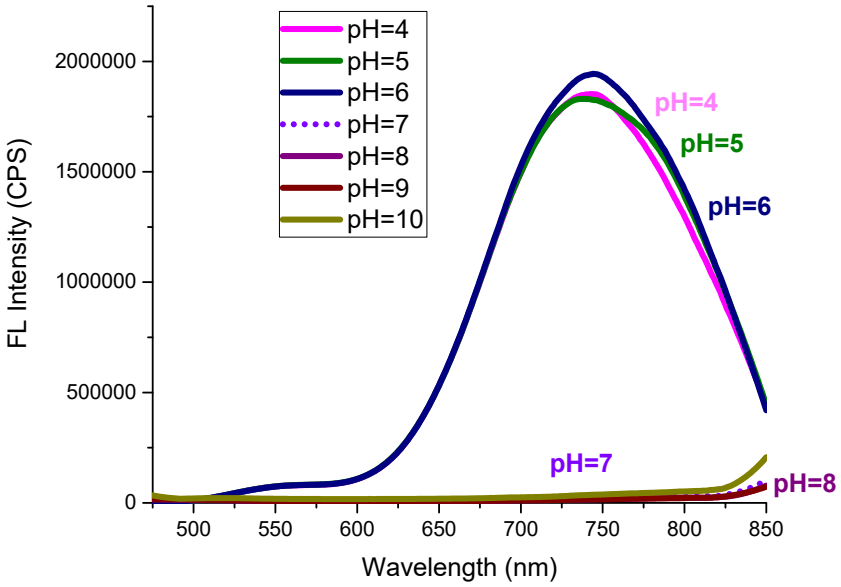


Fig. s25 Fluorescent spectra of **3** (10 μM) pH buffers (λ_{ex}=450 nm).

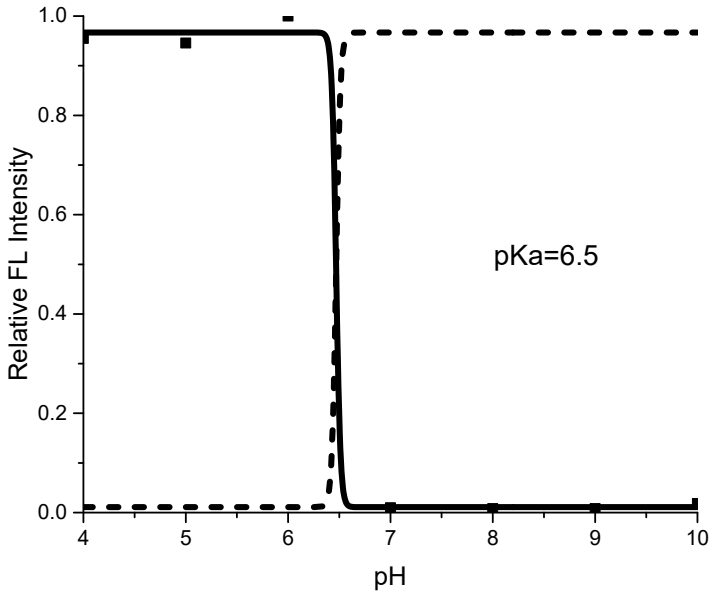


Fig. s26 Response curve of **3** to pH and pKa calculation.

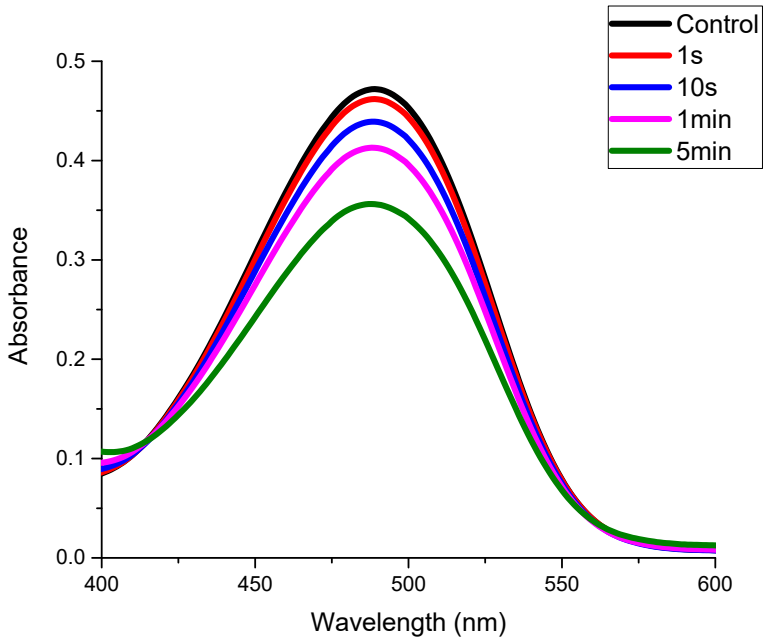


Fig. s27 Photostability test of compound **2** in DCM (10 μM).

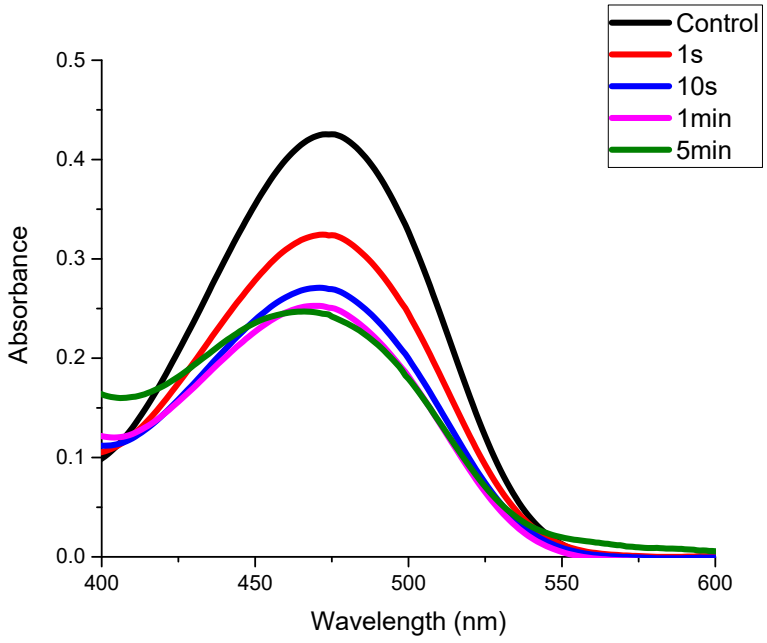


Fig. s28 Photostability test of compound **3** in DCM (10 μM).

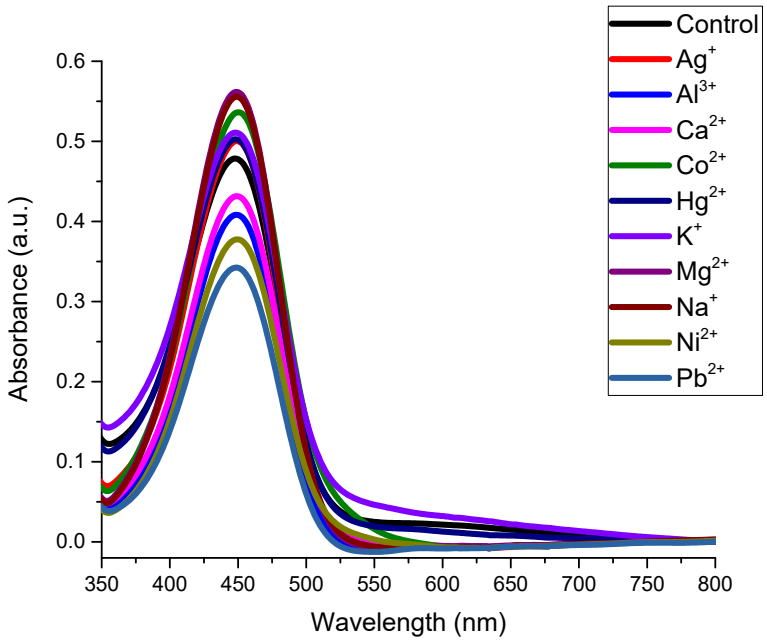


Fig. s29 UV-absorbance spectra of **2** (10 µM) in the presence of one equivalent (10 µM) of different cations in acetonitrile

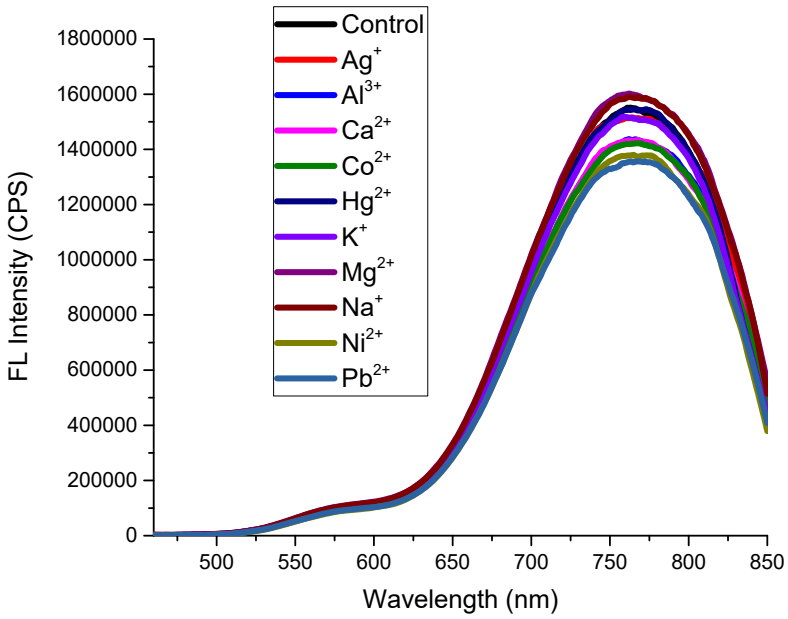


Fig. s30 Fluorescence spectra of **2** (10 µM) in the presence of one equivalent (10 µM) of different cations in acetonitrile

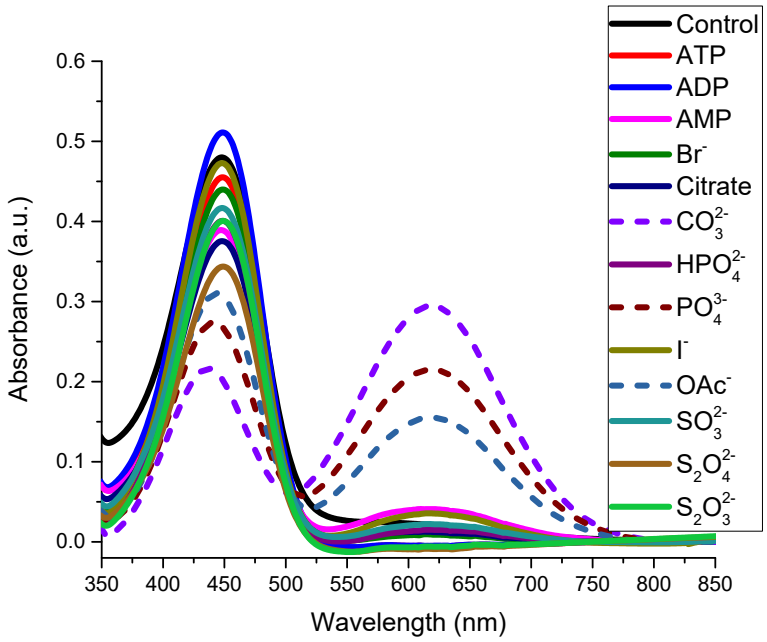


Fig. s31 UV-absorbance spectra of **2** (10 µM) in the presence of one equivalent (10 µM) of different anions in acetonitrile

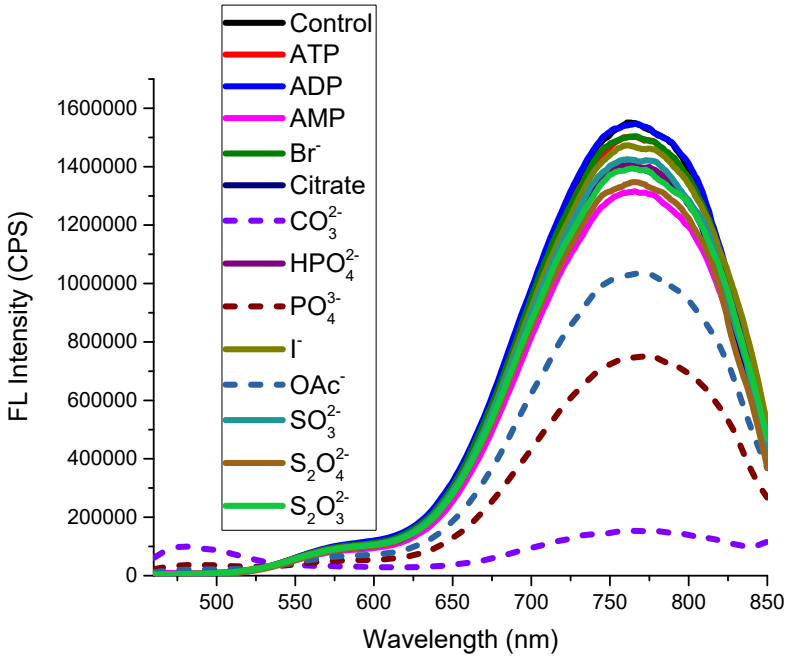


Fig. s32 Fluorescence spectra of **2** (10 µM) in the presence of one equivalent (10 µM) of different anions in acetonitrile

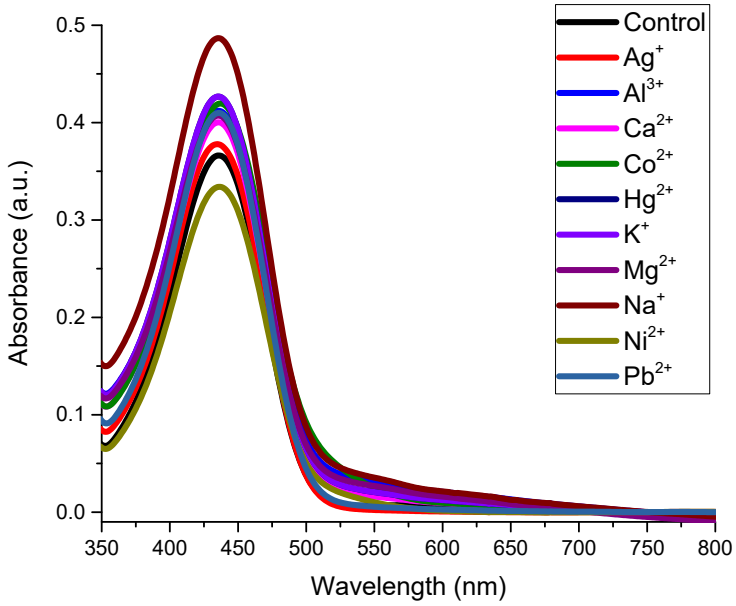


Fig. s33 UV-absorbance spectra of **3** (10 µM) in the presence of one equivalent (10 µM) of different cations in acetonitrile

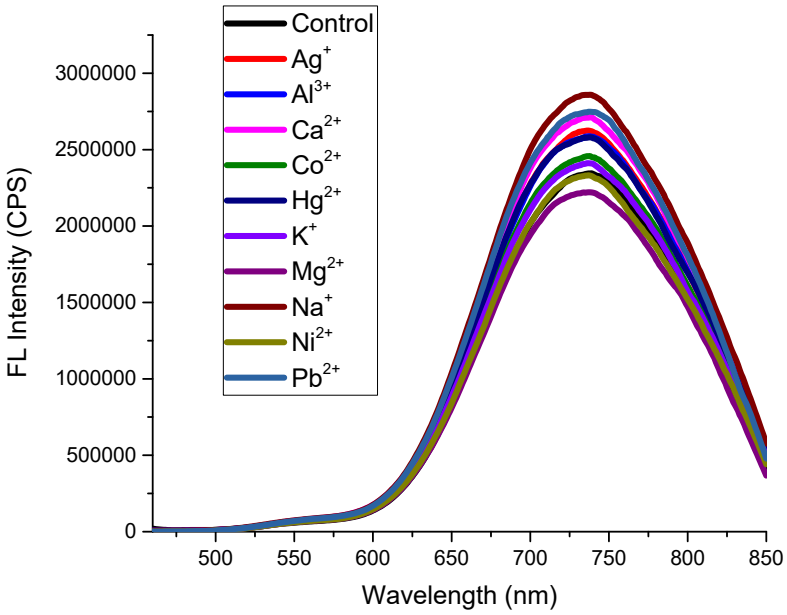


Fig. s34 Fluorescence spectra of **3** (10 µM) in the presence of one equivalent (10 µM) of different cations in acetonitrile

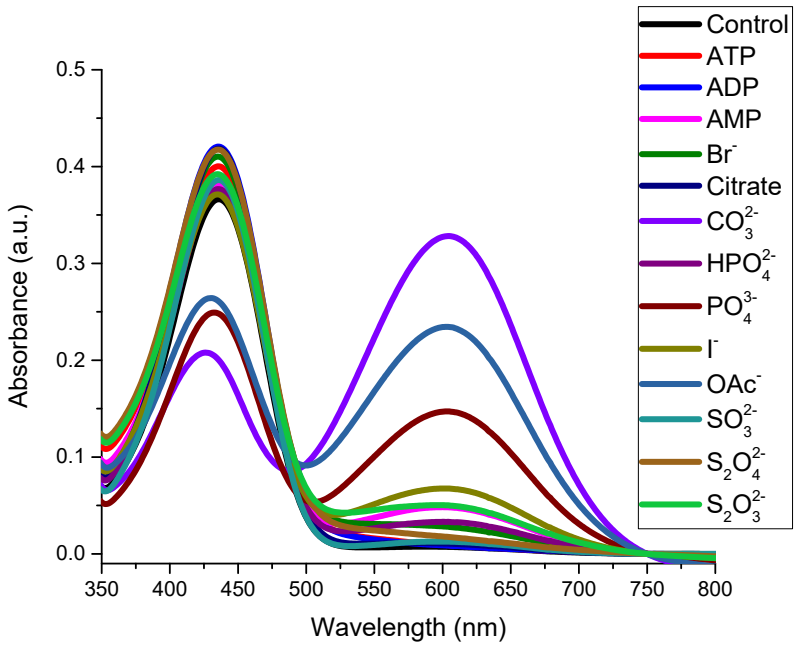


Fig. s35 UV-absorbance spectra of **3** (10 µM) in the presence of one equivalent (10 µM) of different anions in acetonitrile

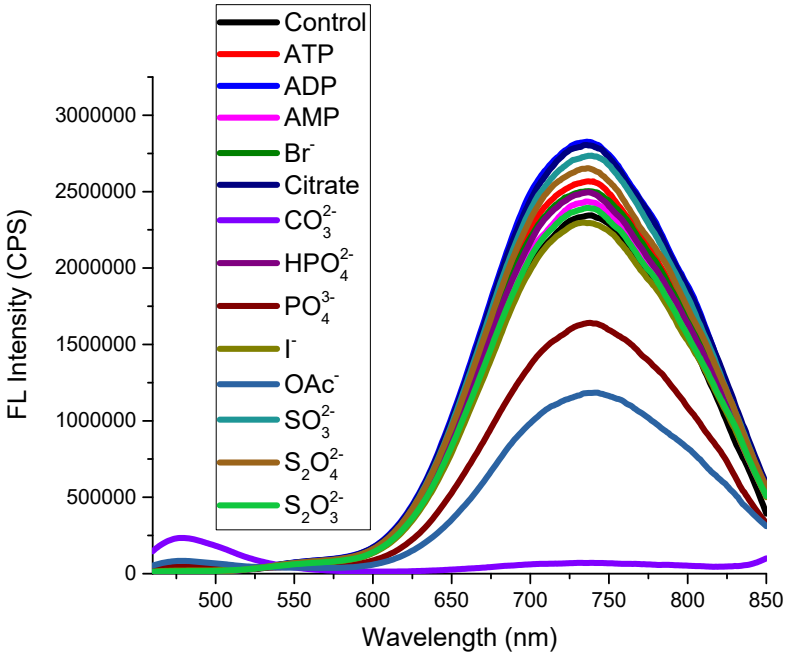


Fig. s36 Fluorescence spectra of **3** (10 µM) in the presence of one equivalent (10 µM) of different anions in acetonitrile

Zebrafish breeding:

Zebrafish (Danio rerio) were maintained in 10-gallon tanks with 28.5 °C water and 12 h light/12 h dark cycle. Zebrafish embryos were obtained by breeding adult wildtype. Zebrafish embryos were obtained by breeding adult wildtype zebrafish. All embryos were maintained in E3 medium (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, and 10^{-5}% methylene blue at 7.5 pH) at 28.5 °C. All animal related procedures were approved by the Institutional Animal Care and Use Committee at The University of Akron (D16-00501).

Zebrafish staining and imaging:

The stock solution of **2** or **3** (1 mM concentration) was prepared in DMSO. All fish embryos were removed from their chorions before staining. Embryos of 72 h post fertilization (hpf) were used in this study. The embryos from the same breeding were stained with either probe **2** or **3** (10 μM for 30 min) and styryl dye 4-Di-2-ASP (50 μM for 1 h), before washing with E3 medium 3 times. Imaging was performed on a Nikon A1 confocal system with a Plan Apo λ 10x and a Plan Apo λ 100x Oil optics with both GaAsP detectors and high sensitivity low noise PMTs for detection. Excitation wavelength for 4-Di-2-ASP was 488 nm with a 595 nm filter and excitation for **2** and **3** was 488 nm with filters at 700 nm. Images were processed using NIS Elements software.

Cell staining and plasmolysis protocol:

E. coli cells were cultured and preceded as desired in reference.[1] When using **2** and **3** to stain E. coli cells, clear fluorescence images were observed with low concentration of dye (100 nM). Imaging was performed on a Nikon A1 confocal system with a Plan Apo λ 100x Oil optics with both GaAsP detectors and high sensitivity low noise PMTs for detection. Excitation wavelength was 488 nm with a 700 nm filter. Images were processed using NIS Elements software.

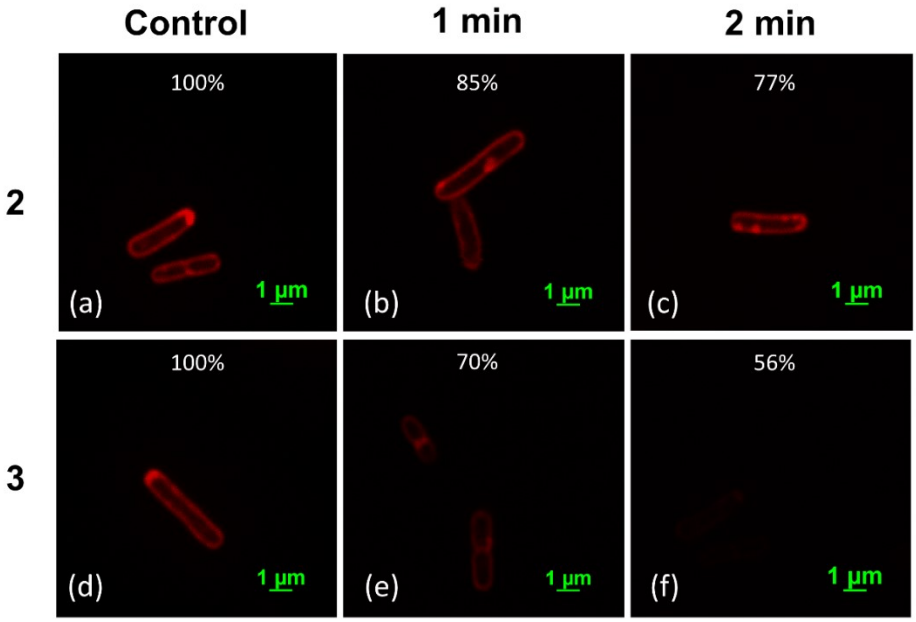


Fig. s37 Fluorescence confocal microscopy images obtained for E. coli cells stained with compound 1 µM **2** (a- c) and **3** (d- f) upon continuous irradiation up to 2 minutes (100× magnification and digital enhanced by 10 times). Excitation/ Emission= 488/ 700 nm.

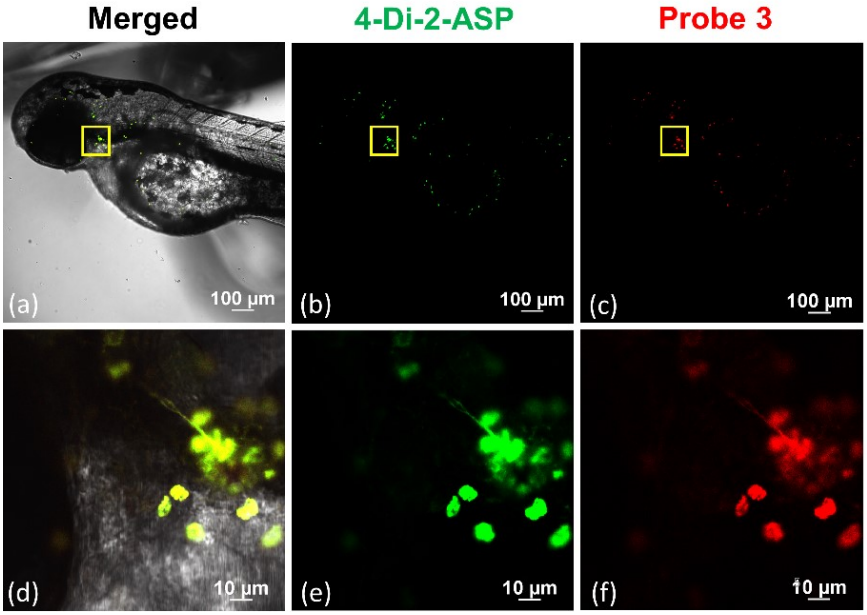


Fig. s38 Confocal images of zebrafish embryos (72 hpf) labeled with 4-Di-2-ASP (b, e) and probe **3** (e, f), merged (a, d) under 10x (first row) and 100x (second raw) magnification. Excitation/ Emission= 488/ 595 nm for 4-Di-2-ASP, and Excitation/ Emission= 488/ 700 nm for probe **3**.

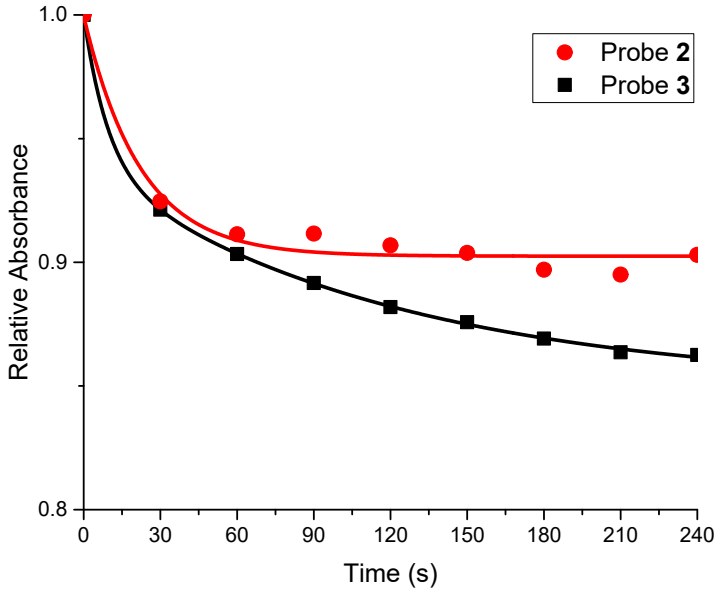


Fig. s39 Comparation of photostability for **2** and **3** in water (10 µM), by monitoring the decay of λ_{abs}.

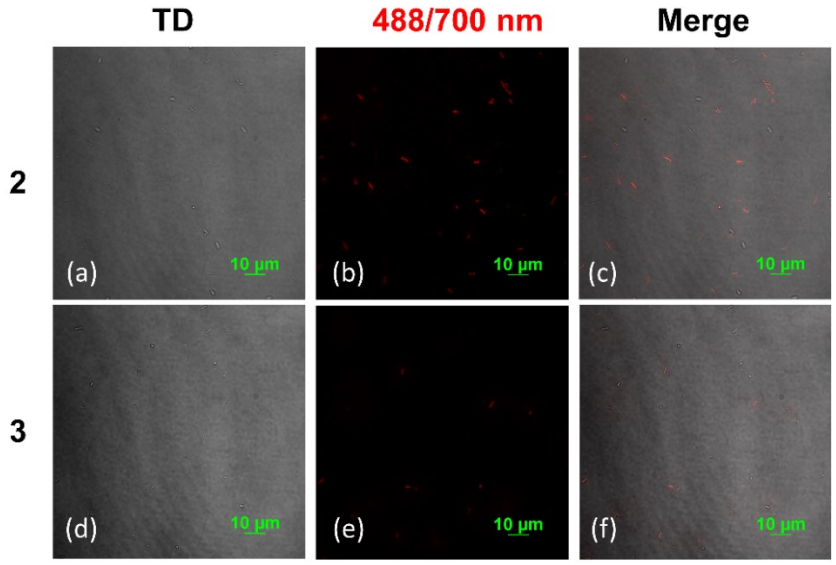


Fig. S40 Fluorescent confocal microscope images of *E. coli* cells stained with 1 µM compound **2** (a- c) and **3** (d- f) in TD (a, d), Excitation/ Emission= 488/ 700 nm (b, e) and overlap (c, f) under magnification of 100x.

Reference

1 A. R. Tuttle, N. D. Trahan and M. S. Son, *Curr Protoc*, 2021, **1**, e20.