Electronic Supplementary Information

Charge transfer based fluorescent probe for selective detection of hydrogen peroxide among different reactive oxygen species

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Instruments and experimental procedures

General information

All reagents were purchased from Aldrich and were used without further purification. Ethanol (AR grade) was used to perform analytical studies. UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length, 1 cm). The fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-FT NMR-AL 300 MHz using $CDCl_3$ as solvent and tetramethylsilane $SiMe_4$ as internal standards. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, d = doublet, q = quartet, br = broad singlet, m = multiplet, dd = doublet of doublet), coupling constants (Hz), integration, and interpretation. Fluorescence quantum yield[1] was determined by using optically matching solution of naphthalene (Φ_{fr} = 0.23 in ethanol) as standard at an excitation wavelength of 400 and quantum yield is calculated using the equation:

$$\Phi_{fs} = \times \frac{1\text{-}10^{-AsLs}}{1\text{-}10^{-ArLr}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

Φ_{fs} and Φ_{fr} are the radiative quantum yields of sample and the reference respectively, A_s and A_r are the absorbance of the sample and the reference respectively, Ds and Dr the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively).

[1] J. N. Deams and G. A. Grosby, *J. Phys. Chem*., 1971, **75**, 991.

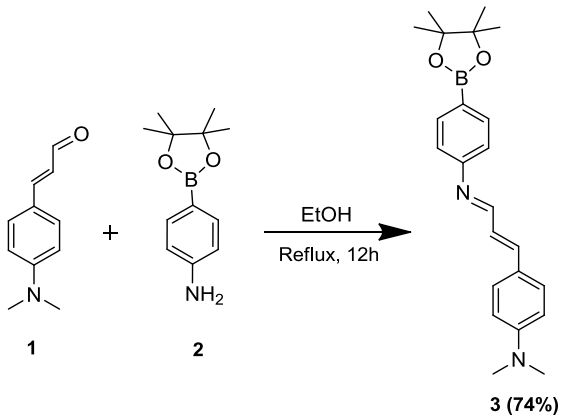
Procedure for metal ion sensing

UV-vis and fluorescence titrations were performed on 5.0 μM solutions of ligand in EtOH or EtOH:H_2O (2:8, v/v). Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively. In titration experiments, each time a 3 ml solution of **3** was filled in a quartz cuvette (path length, 1 cm) and spectra were recorded after incubation with appropriate reactive oxygen species with time.

Procedure for fluorescence imaging

The prostate cancer (PC3) cell lines were incubated with receptor **3** (5 μM in EtOH:H_2O (2:8, v/v) buffered with HEPES, pH = 7.0) in RPMI-1640 medium for 20 min at 37ºC and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **3**. The cells pre-treated with **3** were then treated with hydrogen peroxide (5 μM) in RPMI-1640 medium and incubated for further 20 min at 37ºC and washed with PBS buffer. The cells were imaged by confocal fluorescence microscope OLYMPUS FLUO VIEW FV1000 with excitation wavelength 405 nm. Fluorescence images are recorded at both green (470 ± 20 nm) and red channels (570 ± 20 nm).

Synthetic routes and characteristic data



Scheme 1. Synthesis of compound **3.**

Compound **2**[2] was synthesized according to the literature procedure.

Synthesis of compound - 3:

A mixture of compound **2** (0.13 g, 0.62 mmol) and *N*,*N*-dimethylaminocinnamaldehyde (0.1 g, 0.57 mmol) in ethanol was refluxed for 12 hour. After the completion of the reaction solvent was evaporated and the residue left was crystallized from $CHCl_3/CH_3OH$ to give compound **3** in 74% yield; m.p. 184 °C; IR (KBr) ν_{max} = 1662 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.34 (s, 12 H, CH_3), 3.02 (s, 6 H, CH_2), 6.68 (d, 2 H, *J* = 9 Hz, ArH), 6.88-6.98 (m, 1 H, CH), 7.06-7.15 (m, 3 H, CH and ArH), 7.42 (d, 2H, *J* = 9, ArH) 7.80 (d, 2H, *J* = 9 Hz, ArH), 8.20 (d, *J* = 9 Hz, 1 H, HC=N) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 24.81, 40.06, 83.62, 111.94, 113.98, 120.22, 123.51, 129.09, 130.42, 135.79, 136.34, 145.34, 151.34, 154.76, 162.77. MS calcd. 376.23; found ES+, *m/z*: = 377.23 $[M+H]^+$; $C_{23}H_{29}BN_2O_2$: calcd. C 73.41, H 7.77, N 7.44; Found C 73.27, H 7.52, N 7.59.

[2] M. Kumar, N. Kumar, V. Bhalla, P. R. Sharma and T. Kaur, *Org. Lett.*, 2011, **13**, 1422.

Synthesis of compound - 2:

To a suspension of $[PdCl_2(PPh_3)_2]$ (5.08 g, 39.73 mmol) in dioxane (15 mL) were added 4-bromoaniline (2.5 g, 14.45 mmol), 4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolane (5.08 g, 39.73), and triethylamine (5.83 g, 57.8 mmol) under nitrogen. After stirring for 5 h at 80 ºC, the dioxane was removed under vacuum and the residue so obtained was treated with water, extracted with dichloromethane, and dried over anhydrous Na_2SO_4. The organic layer was evaporated, and the compound was purified by column chromatography using dichloromethane as an eluent to give compound **3** as brown solid in 73% yield; m.p. 160 ºC. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 1.32 (s, 12H), 3.83 (s, 2H), 6.61 (d, 2H, J = 6), 7.62 (d, 2H, J = 6). ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) 69.19, 70.34, 71.00, 76.58, 77.00, 115.15, 118.39, 149.00.

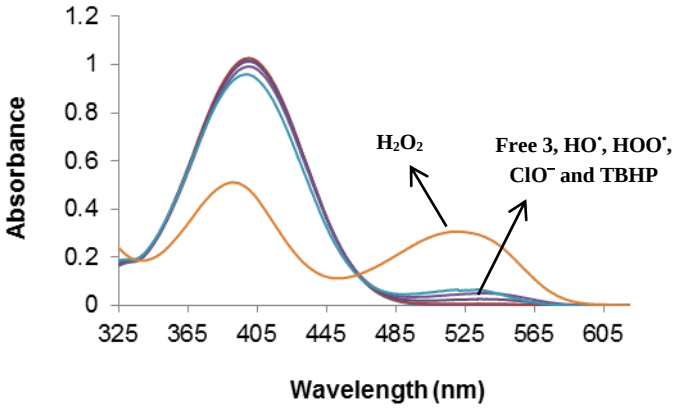


Figure S1. UV-vis spectra of **3** (5 μM, in EtOH) in the presence of various (ROS) reactive oxygen species (10 μM each). Data were given after incubation with the appropriate ROS at 25 °C after 20 min. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively.

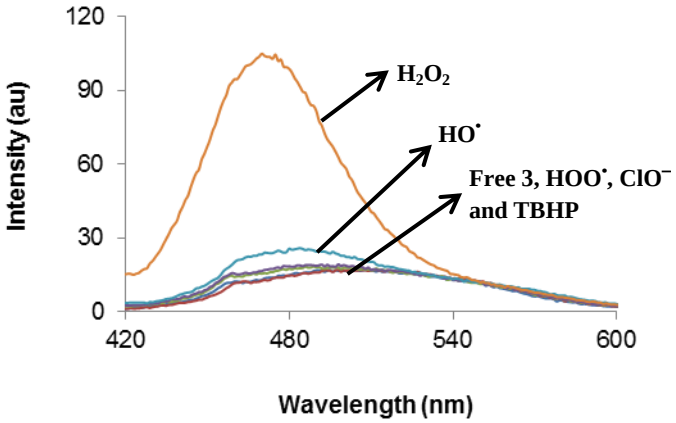


Figure S2. Fluorescence response of **3** (5 μM) in EtOH; λ_{ex} = 400 nm; to various (ROS) reactive oxygen species (5 μM each). (A) Hydroxyl radical (•OH); (B) hypochlorite (OCl^-); (C) *tert*-butoxy radical (•O^{t}Bu); (D) *tert*-butyl hydroperoxide (TBHP), Data were given after incubation with the appropriate ROS at 25 °C after 8 min.

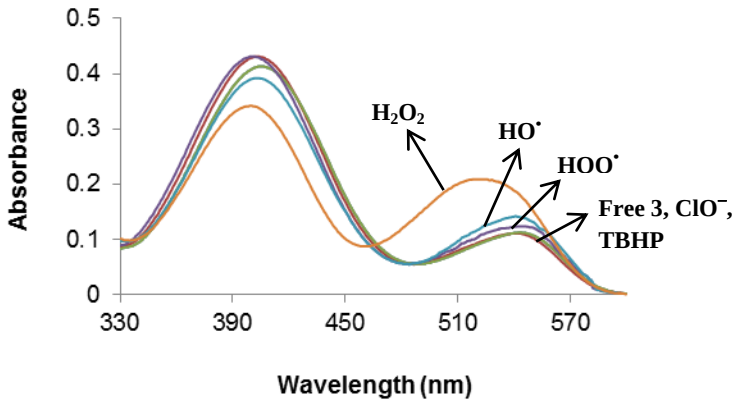


Figure S3. UV-vis spectra of **3** (5 μM) in EtOH:H_2O (2:8, v/v) buffered with HEPES, pH = 7.0; in the presence of various (ROS) reactive oxygen species (10 μM each). Data were given after incubation with the appropriate ROS at 25 °C after 10 min. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively.

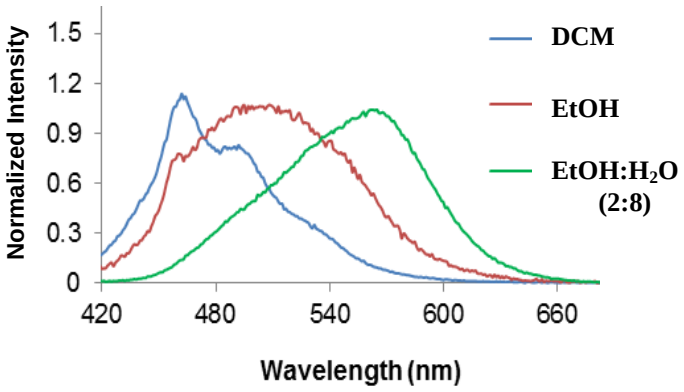


Figure S4. Fluorescence spectra of **3** (5 μM) in different solvent systems; λ_{ex} = 400 nm. In dichloromethane, receptor **3** shows fluorescence emission corresponding to the delocalized excited (DE) state at shorter wavelength (460 nm) with a shoulder at longer wavelength (484 nm) ascribed to the twisted intramolecular charge transfer (TICT) state. Any increase in the polarity of solvents did not affect the position of delocalized excited band. In comparison to delocalized excited band, TICT band shows a large red shift on increasing the polarity of the solvent system. For example, in ethanol a red shift of TICT band (16 nm) was observed in comparison to dichloromethane, while the position of delocalized excited band remains unaffected. On further increase in the polarity of system (aqueous media) more red shift of the TICT band (66 nm) was observed and the presence of delocalized excited band is hardly observed as in this case the delocalized excited band goes underneath the envelop of TICT emission band. Thus, the appearance of red shifted emission band on increasing the polarity of solvent system confirms that the emission of the receptor **3** in aqueous media is due to the twisted intramolecular charge transfer state.

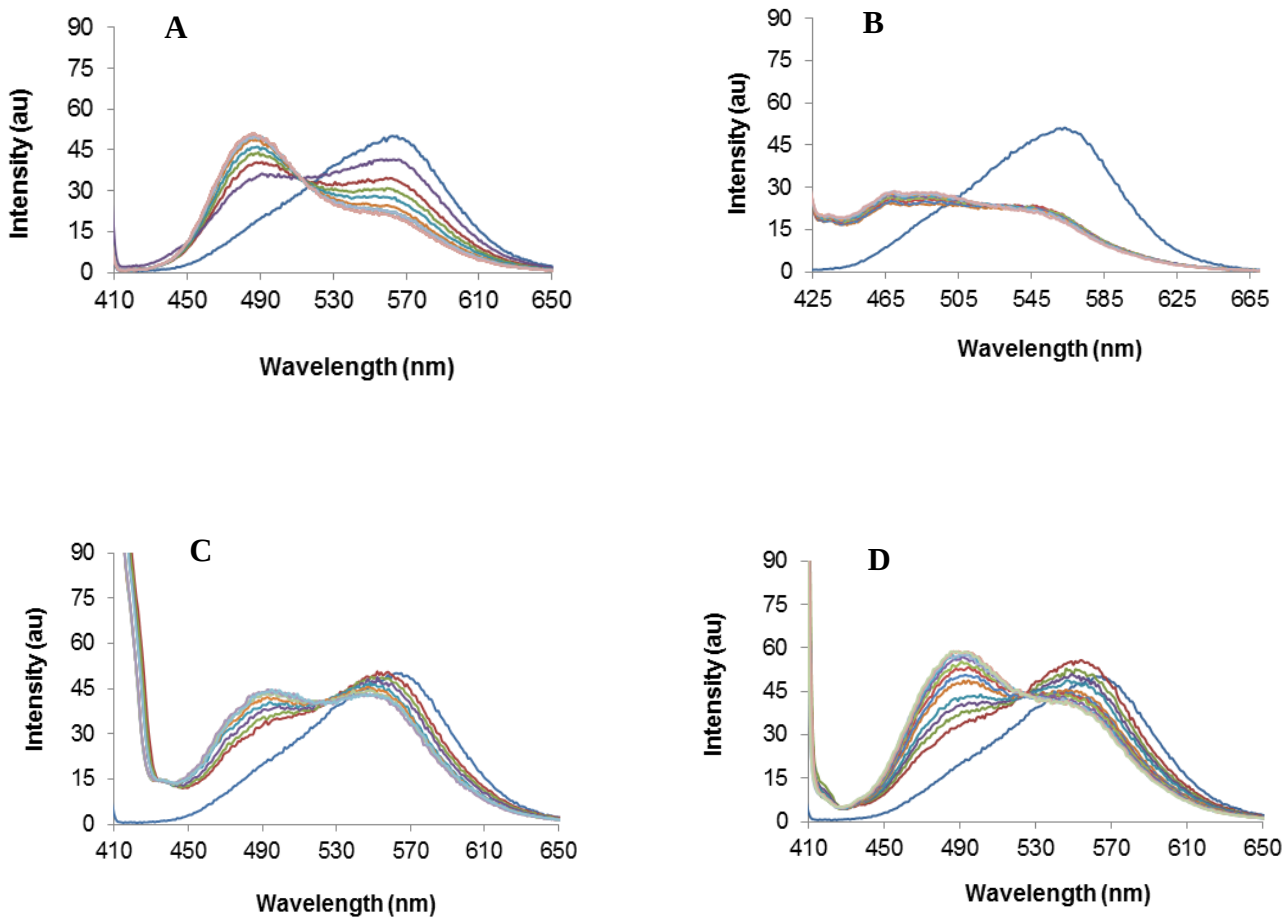


Figure S5. Fluorescence response of **3** (5 μM) in EtOH:H_2O (2:8, v/v) buffered with HEPES, pH = 7.0 (λ_{ex} = 400 nm) to various (ROS) reactive oxygen species (10 μM each). (A) Hydroxyl radical (•OH); (B) hypochlorite (OCl^-); (C) *tert*-butoxy radical (•O^{t}Bu); (D) *tert*-butyl hydroperoxide (TBHP), Data were given after incubation with the appropriate ROS at 25 °C from 0 to 15 min.

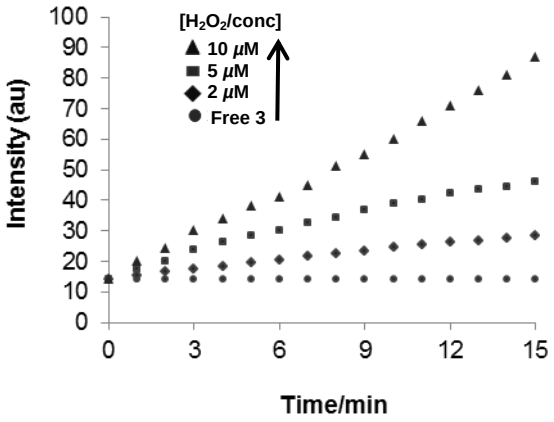


Figure S6. The variation of fluorescence of probe **3** (5 μM) in EtOH:H_2O (2:8, v/v) buffered with HEPES, pH = 7.0; at 566 nm in the presence of different concentrations of H_2O_2; λ_{ex} = 400 nm. The fluorescence spectra were recorded from 0-15 min after the interval of every one minute.

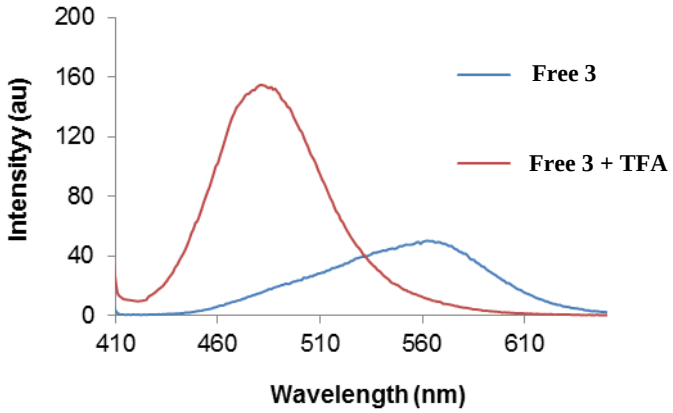


Figure S7. Fluorescence response of **3** (5 μM) in EtOH:H_2O (2:8, v/v) buffered with HEPES, pH = 7.0 (λ_{ex} = 400 nm) in the presence of 3 μL of TFA (Trifluoroacetic acid) Data were given immediately after the addition of TFA. After the addition of TFA, pH of the solution falls to 4.7.

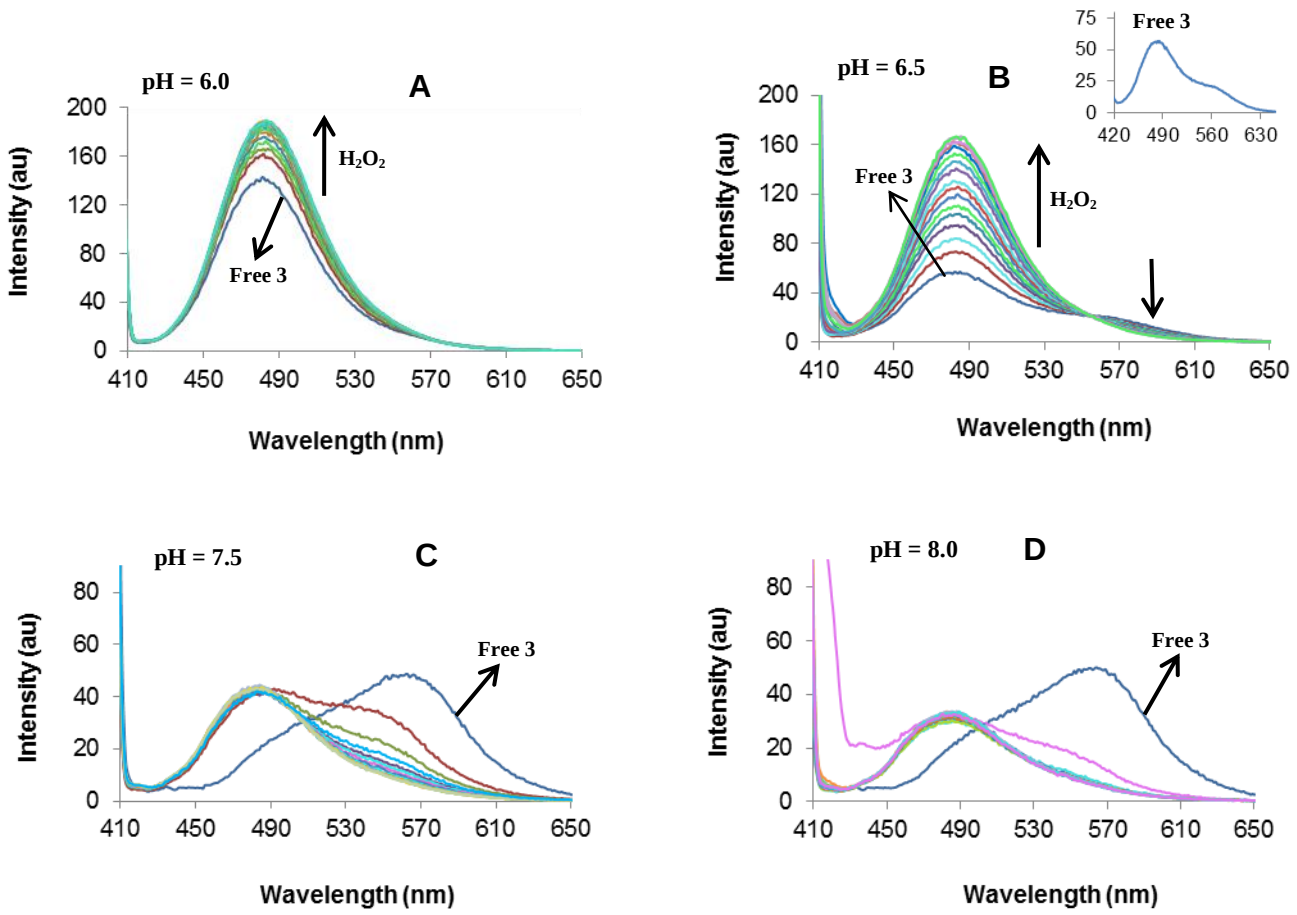
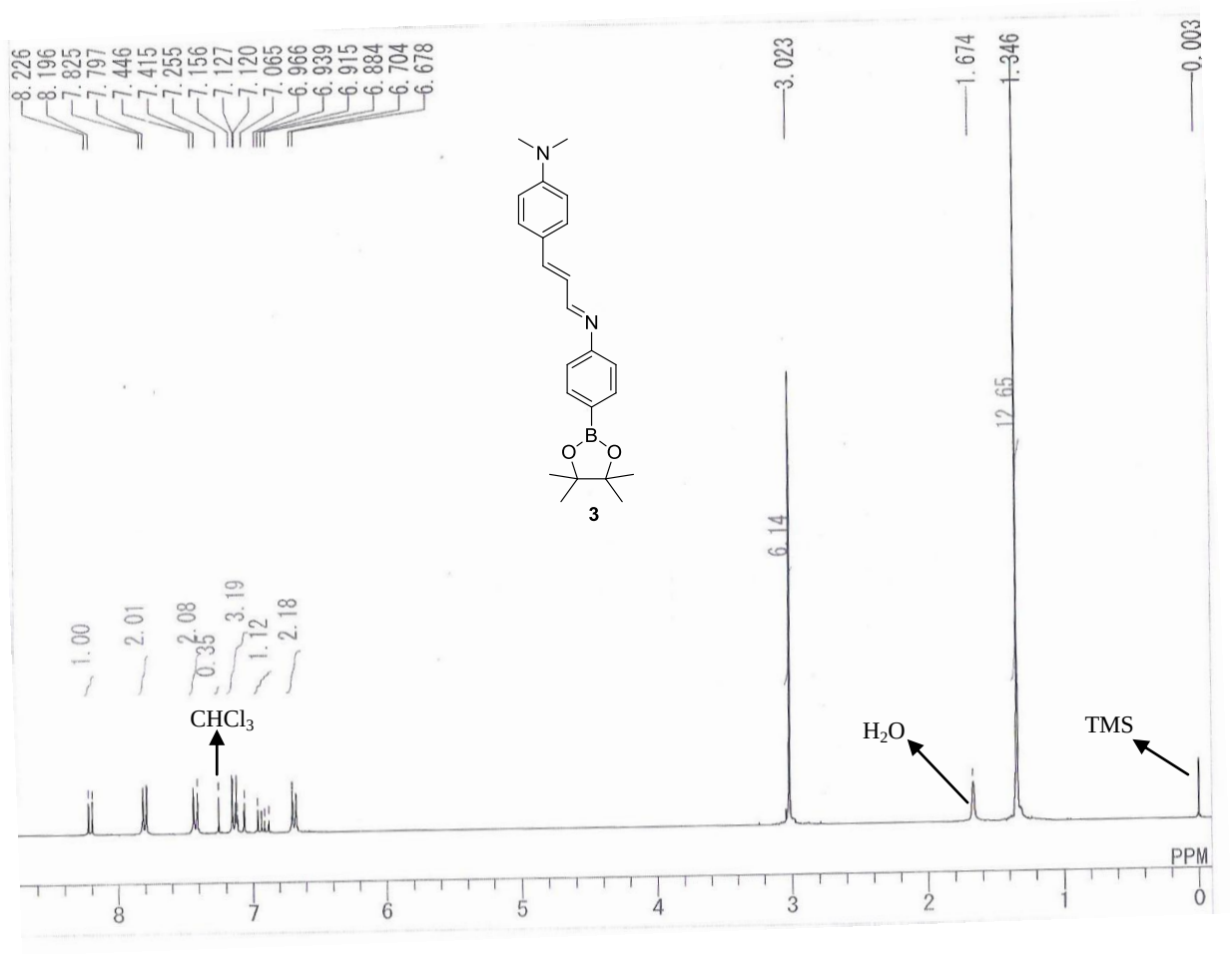
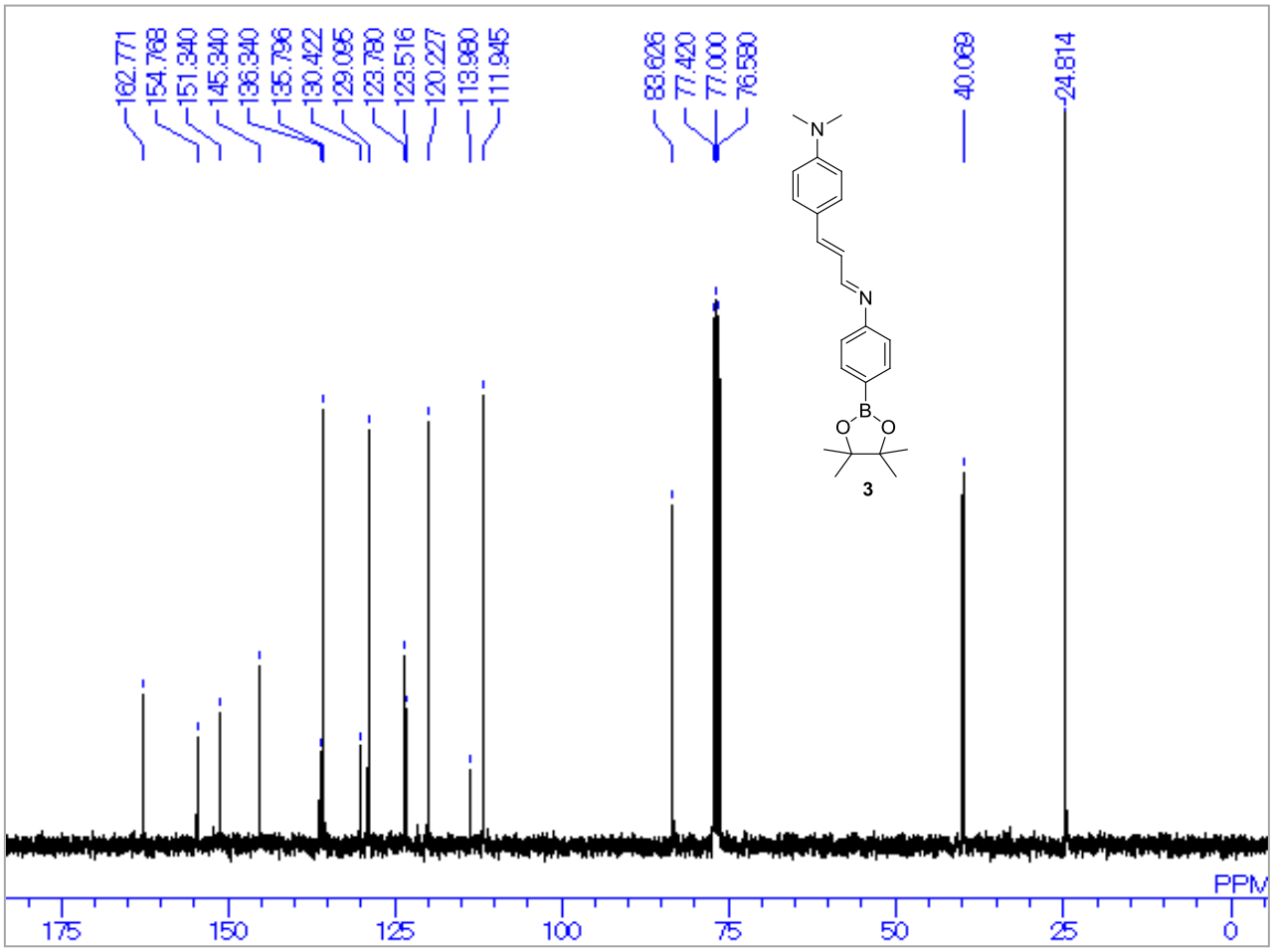


Figure S8. The variation of fluorescence of probe **3** (5 μM) in EtOH:H_2O (2:8, v/v) buffered with HEPES in the presence of H_2O_2 (10 μM); λ_{ex} = 400 nm. The fluorescence spectra were recorded from 0-8 min after the interval of every 30 seconds. The fluorescence spectrum of **3** at pH 6.0 (A) exhibits an emission band at 484 nm corresponding to the delocalized excited (DE) state which upon addition of H_2O_2 undergoes little enhancement at 484 nm. At pH 6.5 (B), **3** exhibits emission (DE) at 484 nm with a shoulder at longer wavelength (566 nm) attributed to the twisted intramolecular charge transfer (TICT) state. However, upon addition of H_2O_2 the emission at 484 nm shows significant fluorescent enhancement while the shoulder at 566 nm undergoes decrease in fluorescence emission (B). At pH 7.5 and 8.0, **3** exhibits emission at 566 nm attributed to the TICT state (C and D). Upon addition of H_2O_2 the emission band at 566 nm undergoes fluorescence quenching with the appearance of delocalized excited band at 484 nm (C and D).

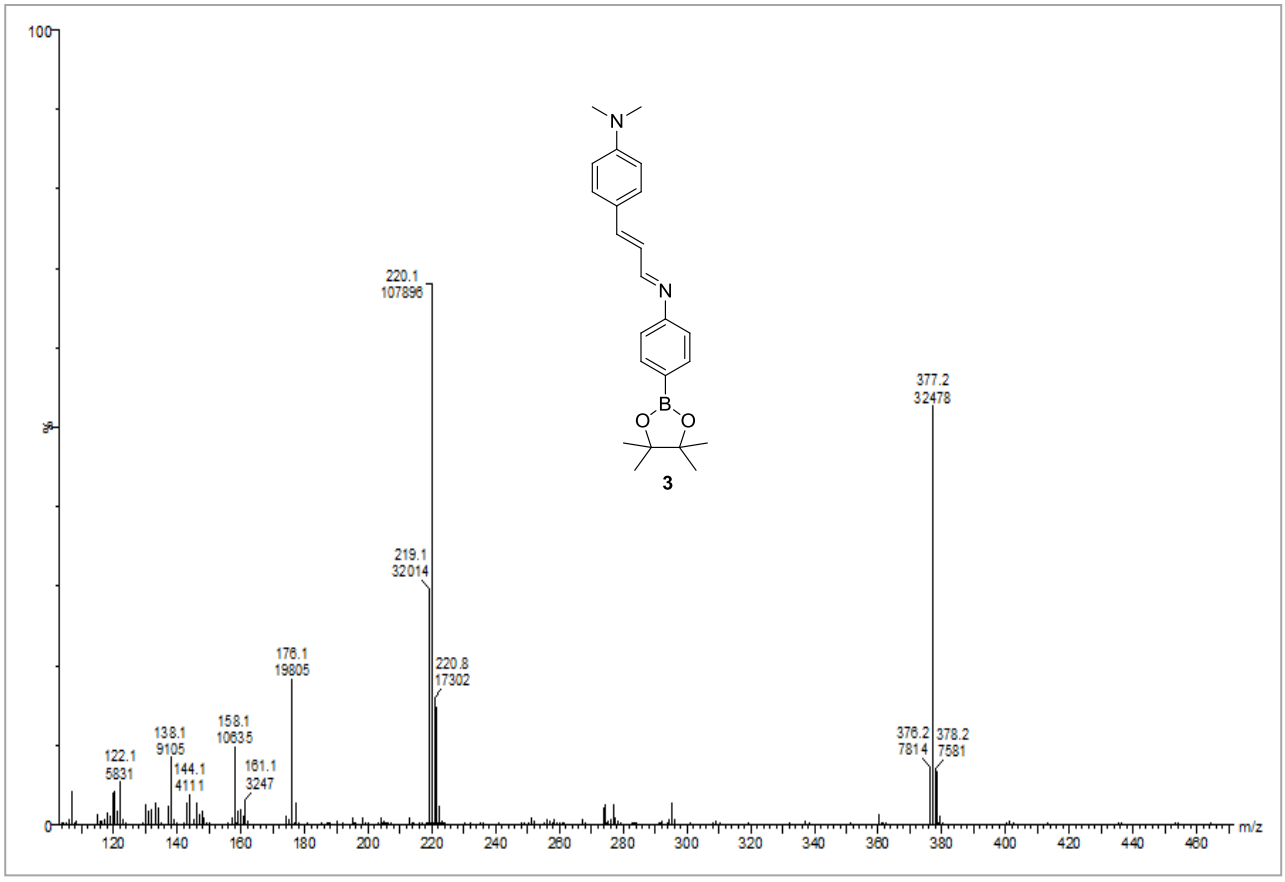
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **3**



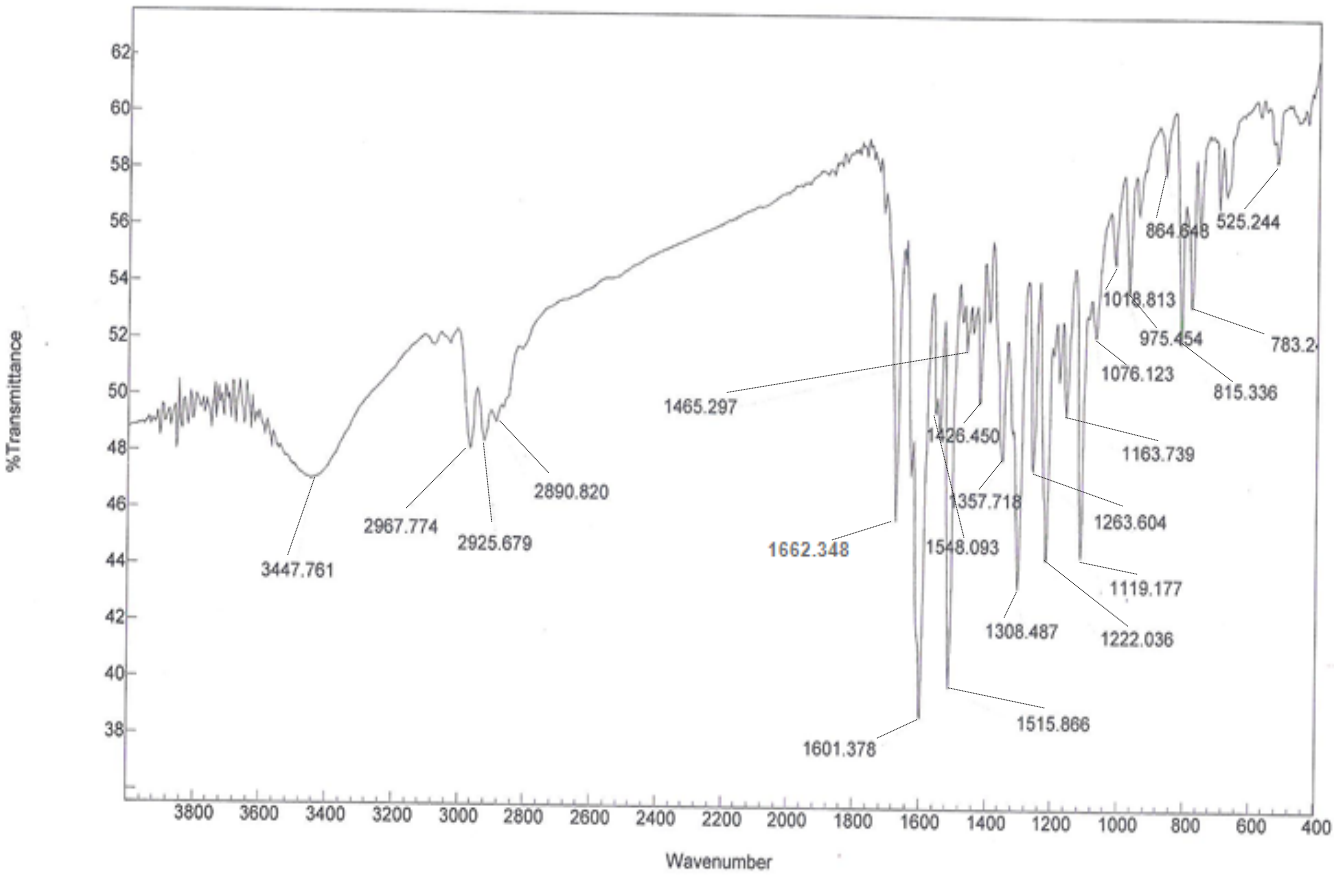
^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **3**



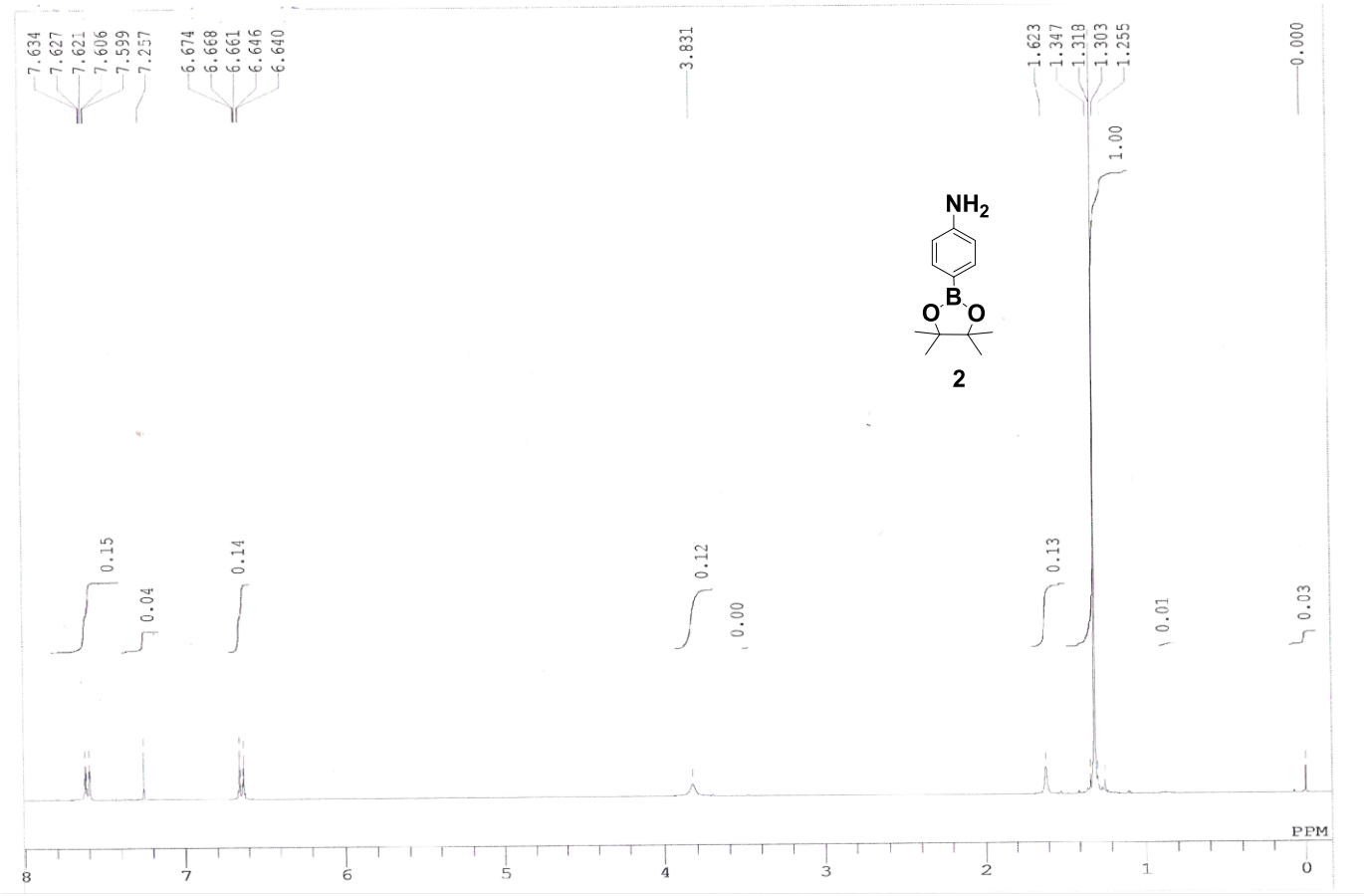
Mass spectrum of **3**



IR spectrum of **3**



^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **2**



^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **2**