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Supplementary Information
for

Intramolecular crossed-benzoin reaction based KCN fluorescent probe in aqueous and biological environments †

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Materials, methods and instrumentations

Resorufin (Aldrich), 2'-formyl [1,1'-biphenyl]-2-carboxylic acid (Aldrich), HATU (Alfa-aesar), DIPEA (Alfa-aesar), DMF (Aldrich), ethyl acetate (LobaChem, India) and hexane (s.d. Fine Chem, India) and sodium sulfate (s.d. Fine Chem, India) were purchased commercially and used without further purification. Column chromatography was performed using silica gel 60 (70-230 mesh) as the stationary phase. Analytical thin layer chromatography was performed using silica gel 60 (precoated sheets with 0.25 mm thickness). Mass spectra were recorded on an IonSpecHiResESI mass spectrometer. NMR spectra were collected on a 400MHz spectrometer (Bruker, Germany).

Synthesis of IND-1

To a solution of 2'-formyl [1,1'-biphenyl]-2-carboxylic acid (226mg, 1.0 mmol) in DMF (10 mL) at 0℃ DIPEA (0.2 mL, 1.12 mmol), resorufin (213 mg, 1.0 mmol) and HATU (400mg, 1.05 mmol) were added. The reaction was continued to rt. for 12h. After completion of reaction, reaction mixture was diluted with water. The organic compound was extracted in EA and dried over anhydrous sodium sulfate. The solution was concentrated and passed through silica-gel column chromatography using EA in hexane (4:1) as eluent, dried to afford **IND-1** as pale yellow solid (257 mg; 61.05 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.89 (s, 1H); 8.25 (d, *J*=7.64 Hz, 2H); 8.01 (d, *J*=7.64 Hz, 2H); 7.72 (m, 4H); 7.62 (m, 1H); 7.40 (m, 3H); 7.27 (s, 1H); 6.91 (m, 2H); 6.28 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): 191.47, 186.24, 164.38, 153.13, 149.31, 148.26, 144.33, 144.19, 140.34, 135.13, 134.76, 133.81, 133.46, 132.89, 131.88, 130.17, 128.97, 128.54, 128.45, 128.26, 118.94, 109.43, 107.19. ESI-MS *m/z* ($M+Na^+$): calcd. 444.080, found 444.084.

Absorption and Fluorescence Studies

All fluorescence and UV/Vis absorption spectra were recorded in RF-5301PC and S-3100 spectrophotometer, respectively. Stock solutions (1mM) of the potassium anion (CN^-, F^-, Cl^-, Br^-, I^-, $CH_3CO_2^-$, HPO_4^{2-}, HCO_3^-, NO_3^-, ClO_4^-, SCN^-) and thiols were prepared in deionized water. The stock solution of probe, **IND-1** was prepared in DMSO. Excitation was carried out at 565 nm with excitation and emission slit widths is 1.5 nm each. The fluorescence experiments were performed with 10μM solutions of **IND-1** in PBS buffer (10 mM, pH 7.4) with 1 % DMSO

and 100μM of various anion and thiols in deionized water at room temperature. The concentrations of each KCN solution were varied, but the total volume was fixed to 3.0 mL.

NMR Titration Study

NMR experiments were performed at Varian instrument (400 MHz). The sample solution (**IND-1**, **IND-1** with CN^-, resorufin and 9,10-Phenanthrenequinone) were prepared in 10% D_2O-DMSO-d_6. The titration experiment was performed with 5 mM solution of **IND-1** in 10% D_2O-DMSO-d_6 and 2.5 mM of KCN in D_2O at room temperature.

Preparation of cell cultures

Human cervical adenocarcinoma epithelial cell (KCLB, Seoul, Korea) were cultured in DMEM (WelGeneInc, Seoul, Korea) supplemented with 10 % FBS (WelGene), penicillin (100 units/ml), and streptomycin (100 μg/mL). Two days before imaging, the cells were passed and plated on glass bottomed dishes (MatTek). All the cells were maintained in a humidified atmosphere of 5 % CO_2/Air (*v/v*) at 37 °C. For labelling, the growth medium was removed and replaced with DMEM without FBS. The cells were treated and incubated with Probe at 37 ℃ under 5 % CO_2 for 10 min. The cells were washed three times with phosphate buffered saline (PBS; Gibco) and then imaged after further incubation in colourless serum-free media for 15 min. The cells were seeded on 24-well plates and stabilized for overnight. **IND-1** was applied to the cells to monitor their uptake as discussed in the main text above. Cytotoxicity of **IND-1**towards a cancer cell line (HeLa cells) was calculated roughly using a standard MTT assay. Cell proliferation was checked after 24 h following a standard manual.

Cell Imaging

Fluorescence microscopy images of labeled cells was obtained with spectral confocal microscopes (Leica TCS SP2) with ×10, ×40 dry and ×100 oil objectives, numerical aperture (NA) = 0.30, 0.75 and 1.30. To obtain images at 570-630 nm range, internal PMTs were used to collect the signals in 8 bit unsigned 512×512 and 1024×1024 pixels at 800 and 400 Hz scan speed, respectively.

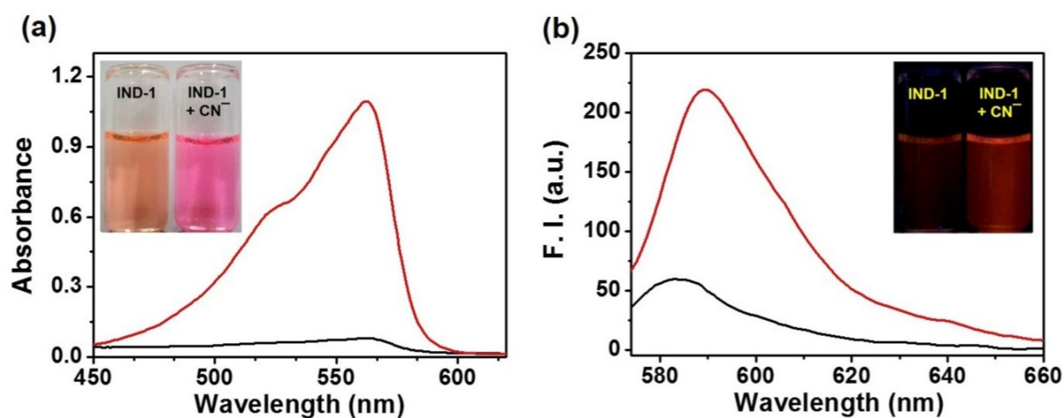


Fig. S1 (a) UV-visible spectra of **IND-1** (10 μM) and **IND-1** with 10 eq. of CN^- (b) Fluorescence spectra of **IND-1** (10 μM) and **IND-1** with 10 eq. of CN^-excitation at 565 nm in PBS buffer (10 mM, pH 7.4) with 1 % DMSO. (Slit widths: ex 1.5 / em 1.5) (inset: photograph of **IND-1** (10.0 μM) and **IND-1** upon addition of 10 eq. of CN^-, which was taken under (a) visible and (b) UV-lamp at 365 nm.)

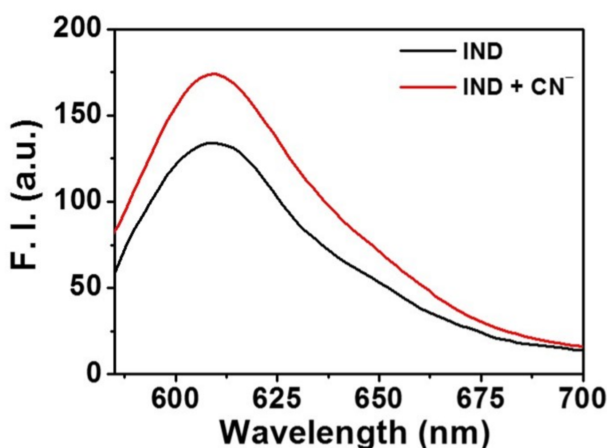


Fig. S2 Fluorescence spectra of **IND-1** and **IND-1** (10.0 μM) with 50 nM of CN^-excitation at 565 nm in human serum with 1 % DMSO.

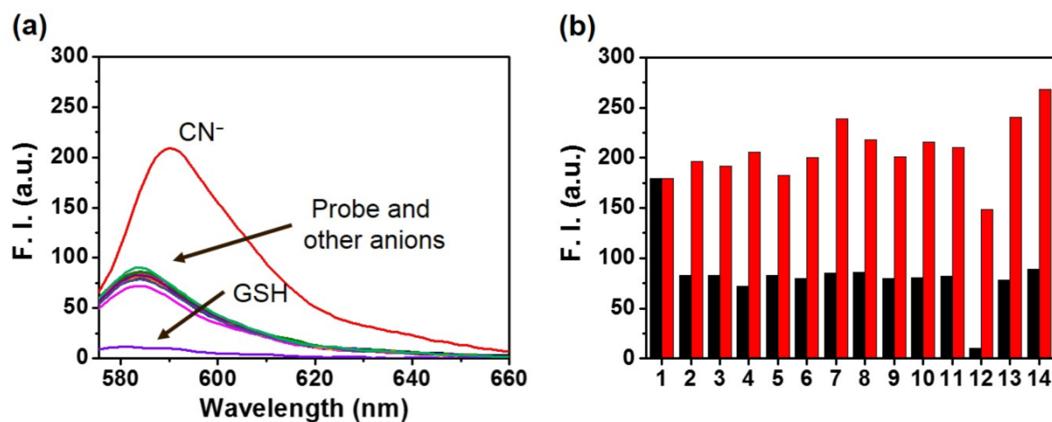


Fig. S3 (a) Fluorescence spectra of **IND-1** (10 μM) in the presence of 10 eq. of various anions and thiols. (b) Red bars represent the emission intensities of **IND-1** upon the addition of various anions and thiols. Black bars represent the emission intensities in the presence of CN^- ions after addition of various metal ions. (1:CN^-, 2:F^-, 3:Cl^-, 4:Br^-, 5:I^-, 6:$CH_3CO_2^-$, 7:HPO_4^{2-}, 8:HCO_3^-, 9:NO_3^-, 10:ClO_4^-, 11:SCN^-, 12:GSH, 13:Cys, 14:Hcy)

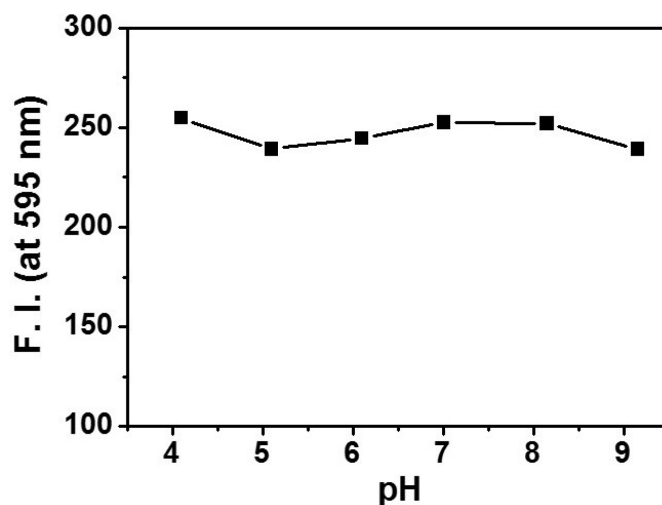


Fig. S4 Fluorescence intensity of **IND-1** (10 μM) with 10 eq. of CN^- in various pH value (pH 4-9) excitation at 565 nm, emission at 595 nm in PBS buffer (10 mM) with 1 % DMSO. (Slit widths: ex 1.5 / em 1.5)

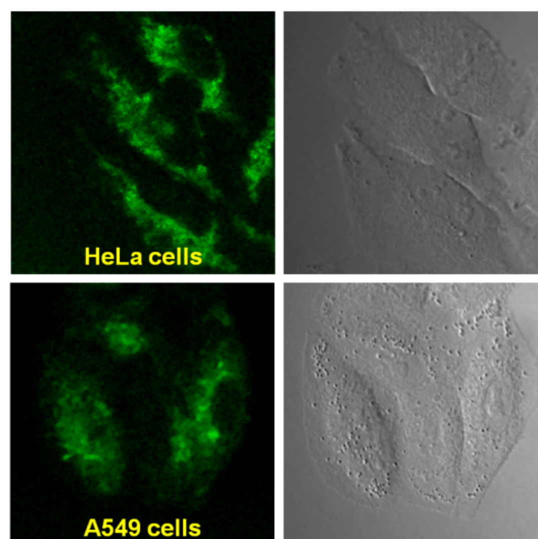


Fig. S5 Confocal microscopy images of HeLa and A549 cells treated with **IND-1**. The left side panels show the confocal microscopy images of HeLa and A549 cells treated with 2 μM of **IND-1** in PBS buffer, The cells were pre-incubated with media containing CN^{-} (0.5 ppm) of 30 min. The right side panels show contrast images. Cell images were obtained using excitation at 543 nm and emission wavelength of 570-630 nm.

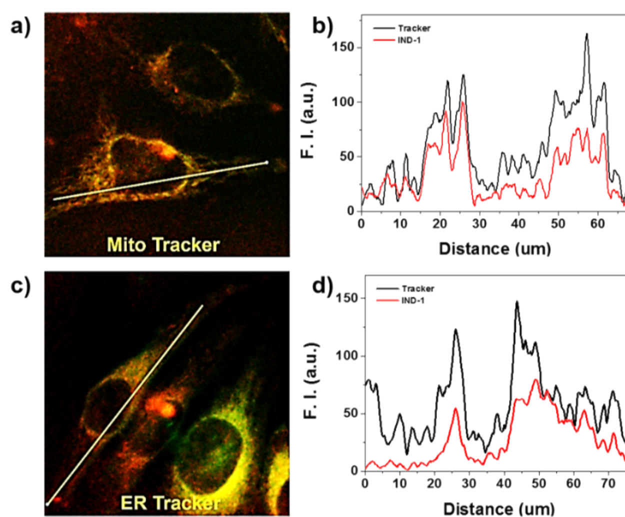


Fig.S6 Confocal microscopic images of co-localized experiment in HeLa cells. (a) Mito tracker green FM (0.5 μM) with **IND-1** (5 μM). (c) ER-tracker green (0.5 μM) with **IND-1** (5 μM). Transverse section of HeLa cells. (b) Profile of fluorescence intensity of region of interest in (a). (d) Profile of fluorescence intensity of region of interest in (c).

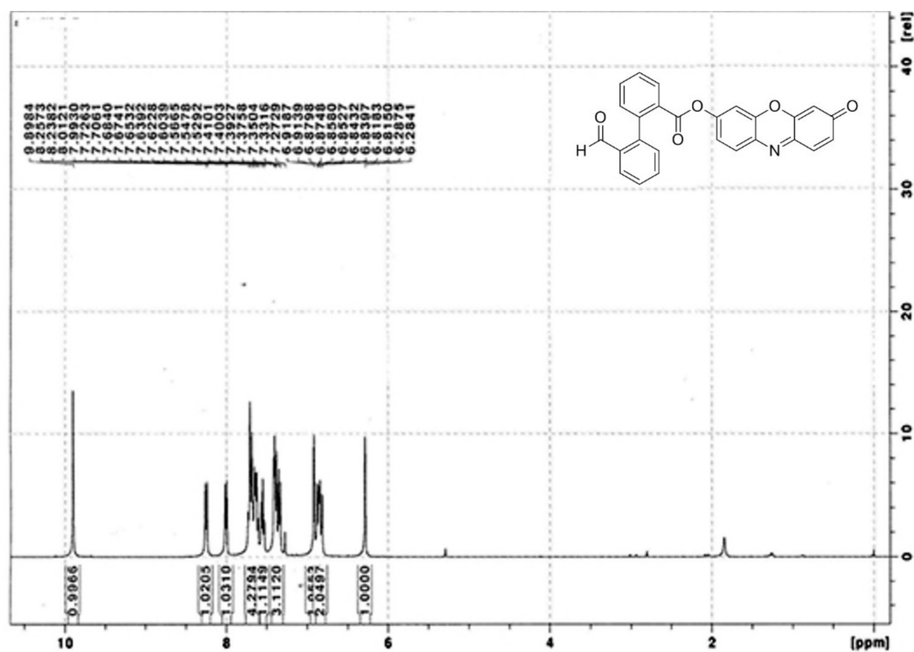


Fig.S7 ^{1}H-NMR of **IND-1** in $CDCl_3$.

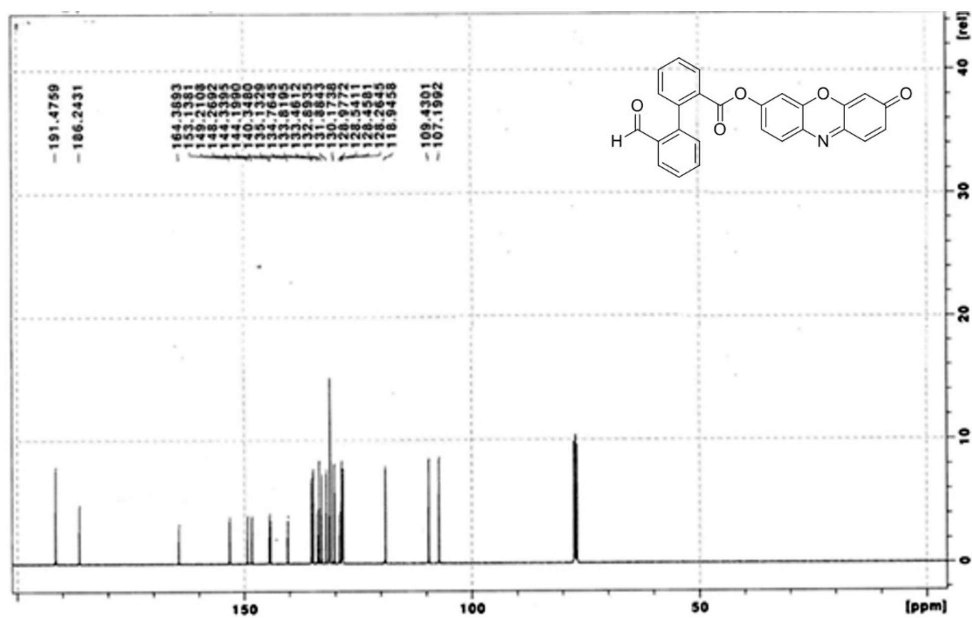


Fig.S8 ^{13}C-NMR of **IND-1** in $CDCl_3$.

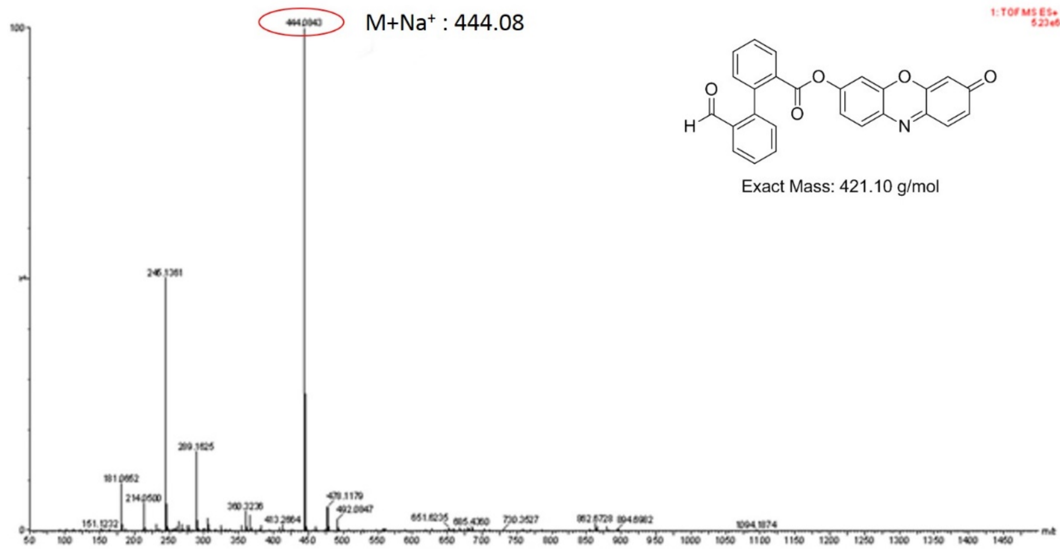
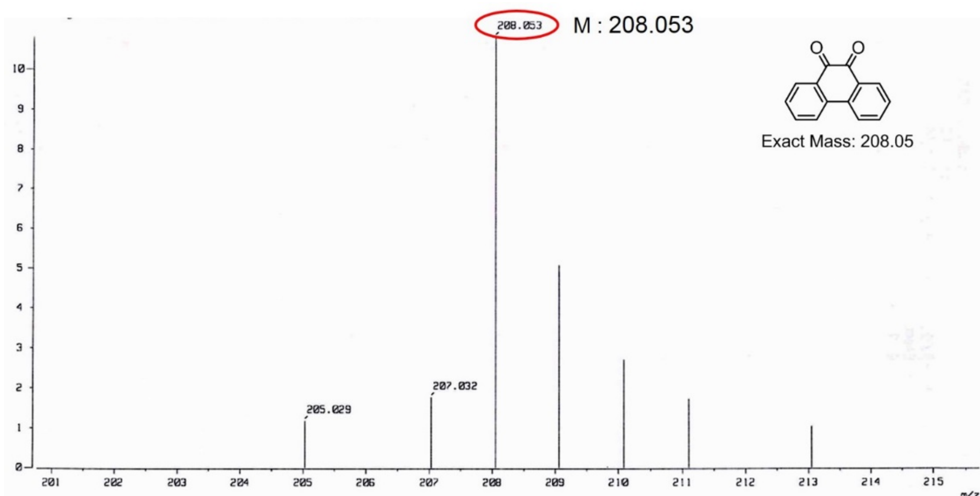


Fig.S9 ESI- MS of **IND-1**.



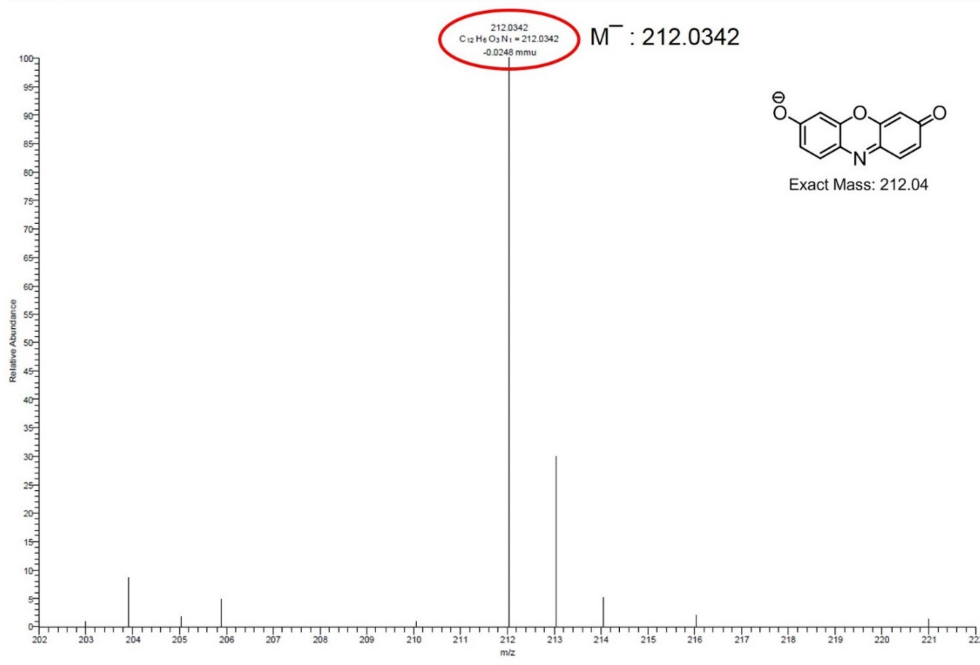


Fig. S10 (a) High resolution EI- MS (b) High resolution FAB-MS of **IND-1** after treatment of KCN (0.5 eq.).