Electronic Supplementary Information (ESI)

An incisive optical recognition of monohydrogen phosphate by a fluorescein based chemodosimeter

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EXPERIMENTAL

1.1 Apparatus:

The IR Spectra for the receptor **FLH** was recorded on JASCO-FTIR Spectrophotometer while ^{1}H NMR and ^{13}C NMR spectra for the same were recorded on a JEOL AL 300 FT NMR Spectrometer. Mass spectrometric analysis was carried out on a MDS Sciex API 2000 LCMS/Brukar Compass data analysis spectrometer. Electronic spectra were recorded at room temperature (298 K) on a UV-1700 pharmaspec spectrophotometer with quartz cuvette (path length= 1.0cm). Emission spectra were recorded on JY HORIBA Fluorescence spectrophotometer.

1.2 Materials:

All reagents for synthesis were purchased from Sigma-Aldrich and were used without further purification.

1.3 General Methods:

All titration experiments were carried at room temperature. All the anions were used as their TBA salts. The ^{1}H NMR spectra were recorded by using tetramethylsilane (TMS) as an internal reference standard. For the ^{1}H NMR titration spectra of **FLH**, 5×10^{-3}Msolutions were prepared in DMSO-d_6 while the stock solution of HPO_4^{2-} was prepared in DMSO-d_6. For UV-visible/fluorescence titration experiments, the solutions of cations were prepared in aqueous medium. Chloride salt of metal ions was used for solution preparation. Due to insufficient solubility of **FLH** in water, its stock solution of 0.25 M was prepared in DMSO which was used for fluorescence titration experiment in EtOH: water (3: 2, v/v)at 1.0μM concentration through dilution.

1.4. Materials and Instrumentation

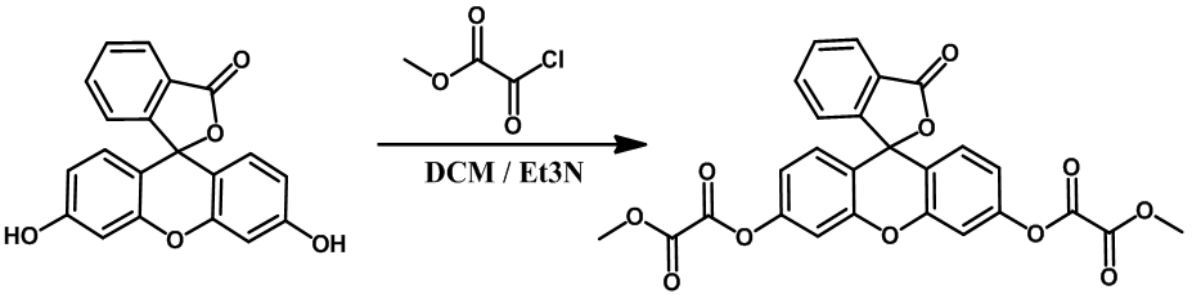
All solvents and reagents (analytical and spectroscopic grade) were obtained from Sigma-Aldrich and were used as received. Solutions of various anions were prepared as their TBA salts.

The chemical shifts (δ) are reported in ppm, relative to tetramethylsilane [$Si(CH_3)_4$]. The UV-vis. absorption spectra were recorded at 25°C using a UV-1700 pharmaspec spectrophotometer. Emission spectra were recorded on Varian Cary Eclipse Fluorescence spectrophotometer and JY HORIBA Fluorescence spectrophotometer. The IR Spectra were recorded on JASCO-FTIR Spectrophotometer while ^{1}H NMR spectra were recorded on JEOL AL 300 FT NMR Spectrometer. Mass spectrometric analysis was carried out on a MDS Sciex API 2000 LCMS spectrometer.

Synthesis of Receptor FLH

To the mixture of fluorescein (0.15g, 0.45mmol) and triethylamine (0.2mL) in 15 mL anhydrous dichloromethane (CH_2Cl_2) at 0°C; methylchlorooxoacetate (0.5 mL, in 5 mL of CH_2Cl_2) was added drop wise with constant stirring over the time period of 30 min. The reaction mixture was warmed at room temperature and was further stirred overnight (**Scheme 1**). The solution was diluted with CH_2Cl_2 (30 mL), washed with brine (30 mL × 2), and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuum to obtain a crude solid mixture. Finally, the target compound **FLH** was isolated as a yellow solid (70% yield) by silica chromatography eluting with CH_2Cl_2. The purity of **FLH** was checked by thin layer chromatography (TLC). The probe **FLH** was fully characterized by ^{1}H &^{13}C NMR, IR along with ESI-MS spectrometry (**ESI; Figure 1 to 4**).

Spectroscopic characterization data of FLH: Yield: 64%; **IR/cm^{-1}**: 3401, 3102, 2924, 2854, 1538, 1523, 1766, 1435, 1364, 1109, 1090, 925, 820; ^{1}H NMR: (**300 MHz, DMSO-*d*$_6$, TMS**): δ = 8.97 (d, 1H, ArH), 7.8-7.7 (m, 1H, ArH), 7.44 (d, 2H, ArH), 7.07, 6.96 (d,d 3H, ArH), 3.87 (s, 3H, CH_3-); **^{13}C NMR (75 MHz, DMSO-*d*$_6$, TMS**): δ =172.40, 169.29, 166.21, 156.53, 154.66, 151.29, 150.72, 129.25, 125.33, 124.96, 123.92, 117.91, 116.93, 110.09, 102.21, 80.62; **ESI-MS**: m/z Calculated for $C_{26}H_{15}NO_9S$ [M] = 504, Found [M+1] = 505.2.

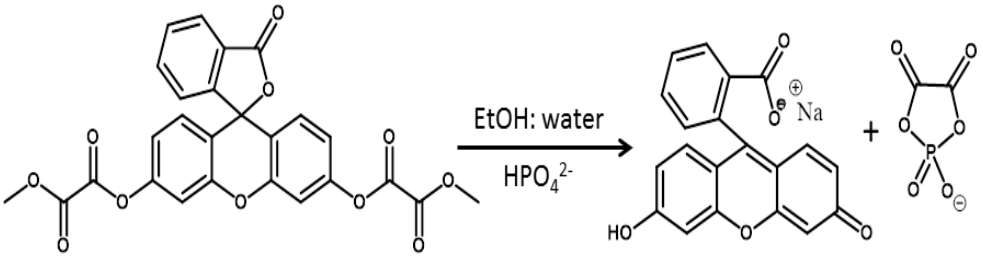


Scheme 1. Synthesis of receptor **FLH**.

Synthesis of FLHP

Mono basic phosphate adduct of **FLH** i.e. **FLHP** was synthesized by adding 3 mL aqueous solution of HPO_4^{2-} (1.5 mmol) slowly to a magnetically stirred 10 mL ethanol: water (3: 2, v/v) solution of **FLH** (0.5 mmol) (**Scheme 2**). The mixture was further stirred at room temperature for ~2 hours where by a yellowish precipitate was formed. The same was filtered and washed several times with diethyl ether and finally dried under vacuum over anhydrous $CaCl_2$.The probe **FLHP** was fully characterized by ^1H &^{13}C NMR, IR along with ESI-MS spectrometry (**ESI; Figure 5 to 8**).

Spectroscopic characterization data of complex FLHP: Yield: 40%; **IR/cm^{-1}**: 3427, 2963, 2876, 2169, 1707, 1634, 1575, 1523, 1486, 1468, 1421, 1387, 1345, 1226, 1209, 1167, 1105, 1033, 882, 740; **^{1}H NMR: (300 MHz, DMSO-*d*$_6$, TMS)**: δ = 10.12 (s, 1H), 8.12 (d, 1H, Ar), 8.02-7.98 (m, 3H, Ar), 7.82-7.74 (m, 2H, Ar), 7.71 (s, 1H, Ar), 7.38-6.51 (m, 3H, Ar), 3.91 (s, 3H, OCH_3); **^{13}C NMR (75 MHz, DMSO-*d*$_6$, TMS)**: δ = 211.68, 168.29, 159.67, 156.50, 154.68, 152.06, 151.44, 151.20, 150.76, 135.46, 130.38, 130.04, 129.11, 128.76, 128.08, 125.83, 124.59, 123.87, 100.00, 109.83, 109.43, 105.24, 51.90; **ESI-MS**: m/z Calculated for $C_{20}H_{11}NaO_5$ [M] = 354 found [M-1] = 353.1.



Scheme 2. Synthesis of **FLHP**.

Figure and Captions

Figure S1: ^{1}H NMR spectrum of **FLH** (in DMSO–d_6):

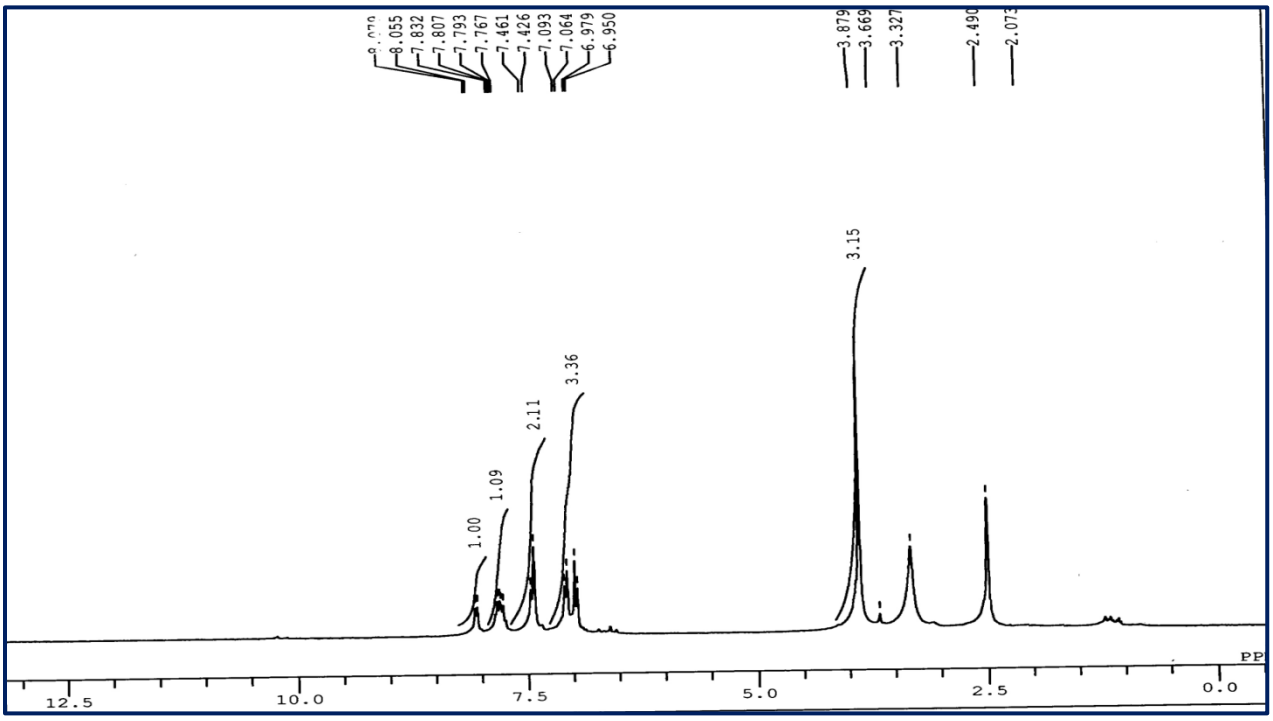


Figure S2: ^{13}C NMR spectrum of **FLH** (in DMSO–d_6):

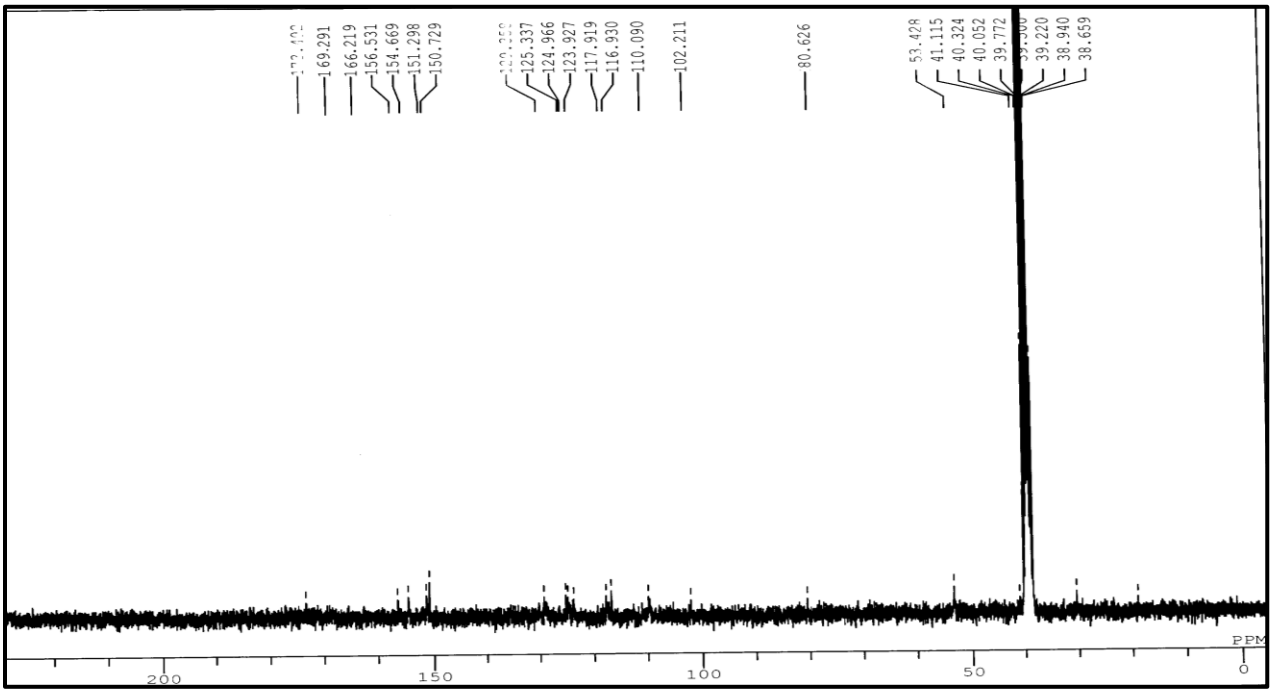


Figure S3: IR spectrum of **FLH**:

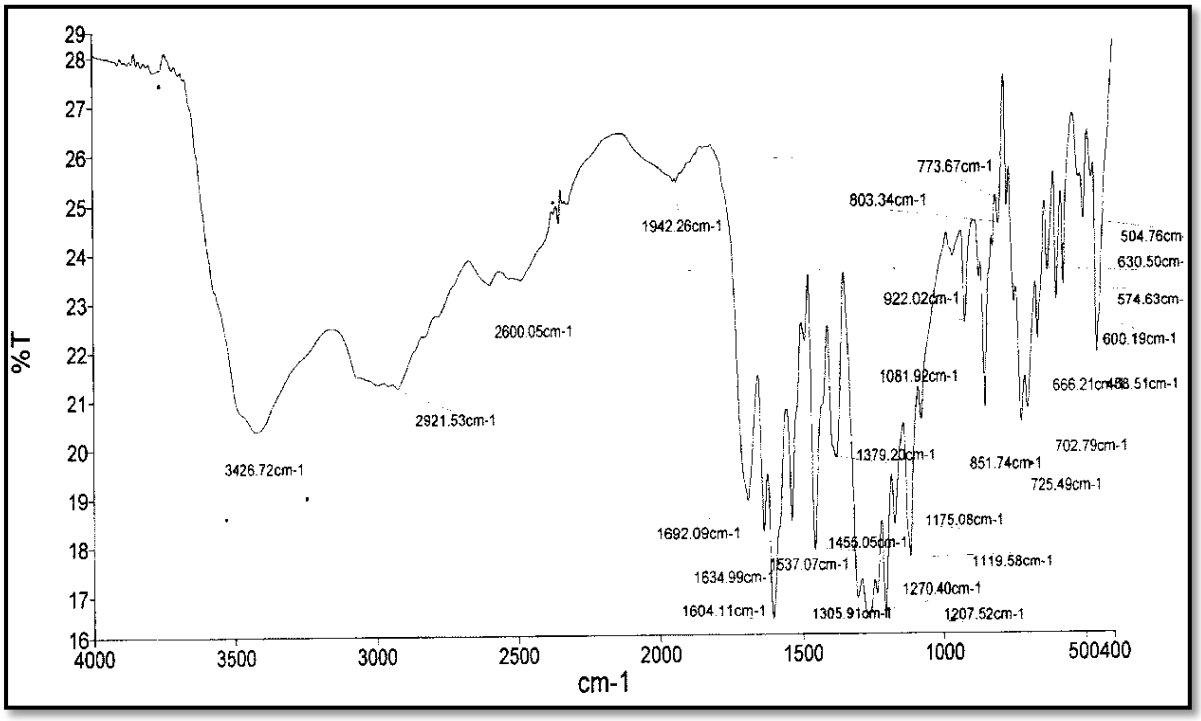


Figure S4: Mass spectrum of **FLH**:

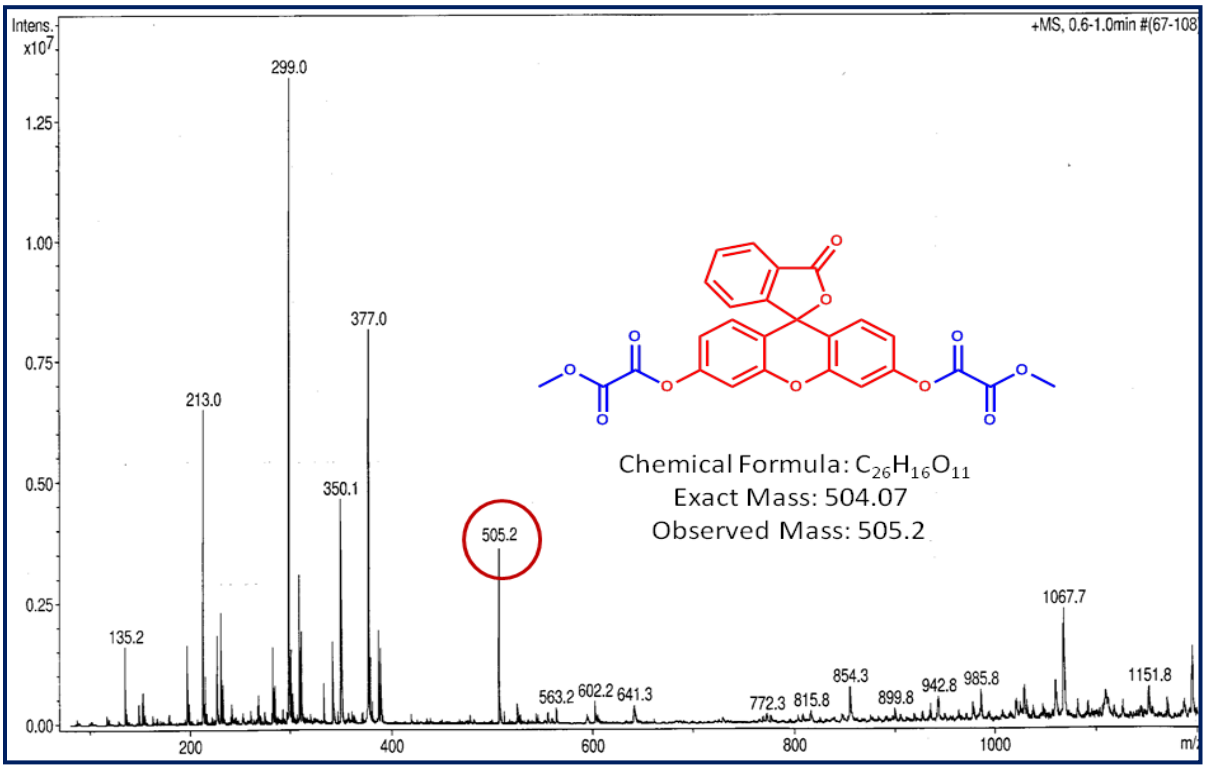


Figure S5: ^{1}H NMR spectrum of **FLHP** (in DMSO–d_6):

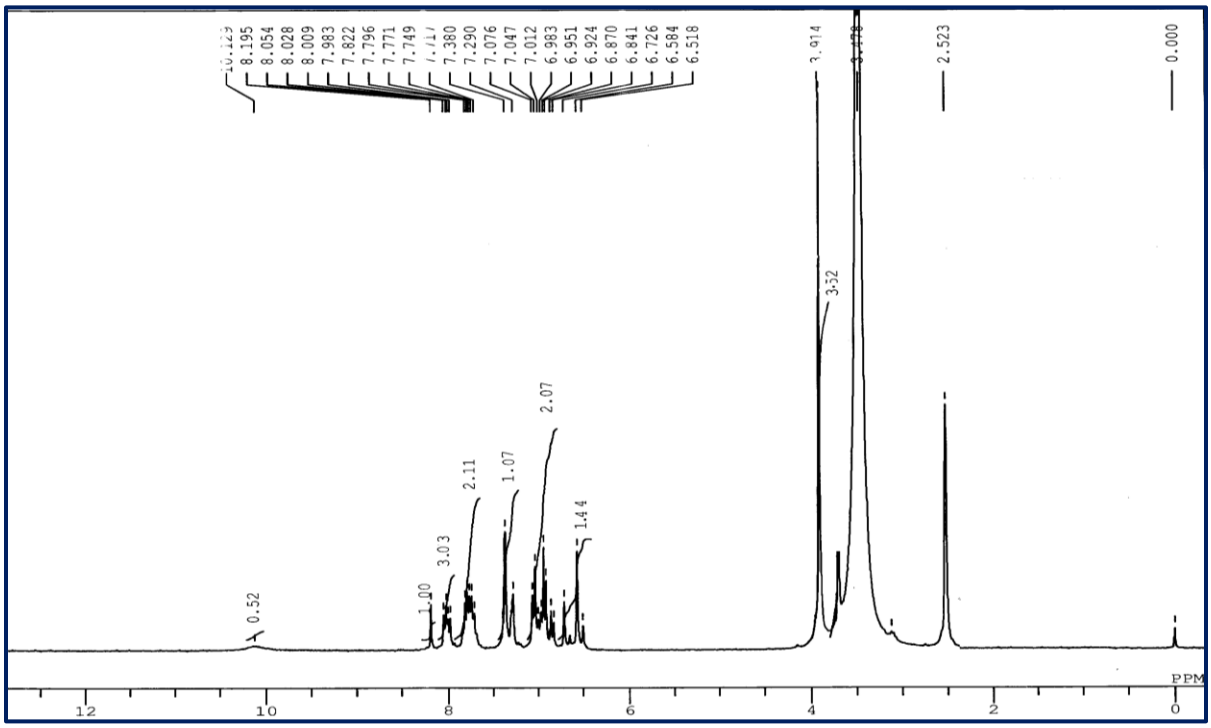


Figure S6: ^{13}C NMR spectrum of **FLHP** (in DMSO–d_6):

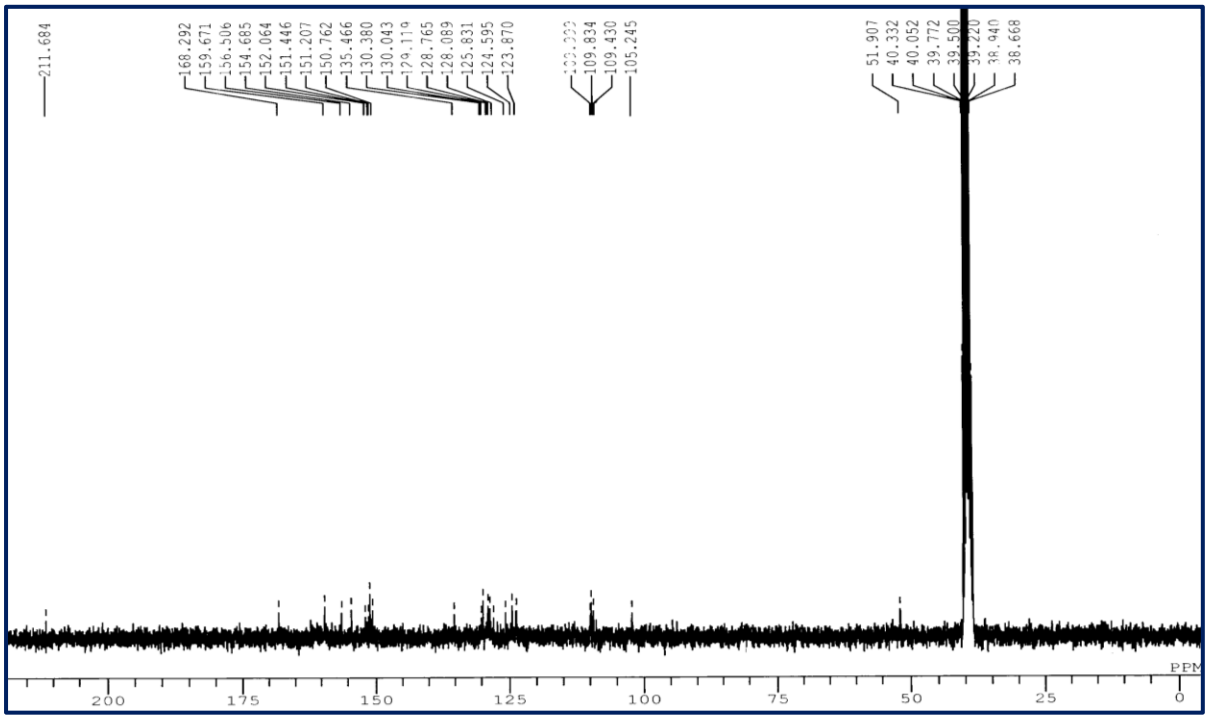


Figure S7: IR spectrum of **FLHP**:

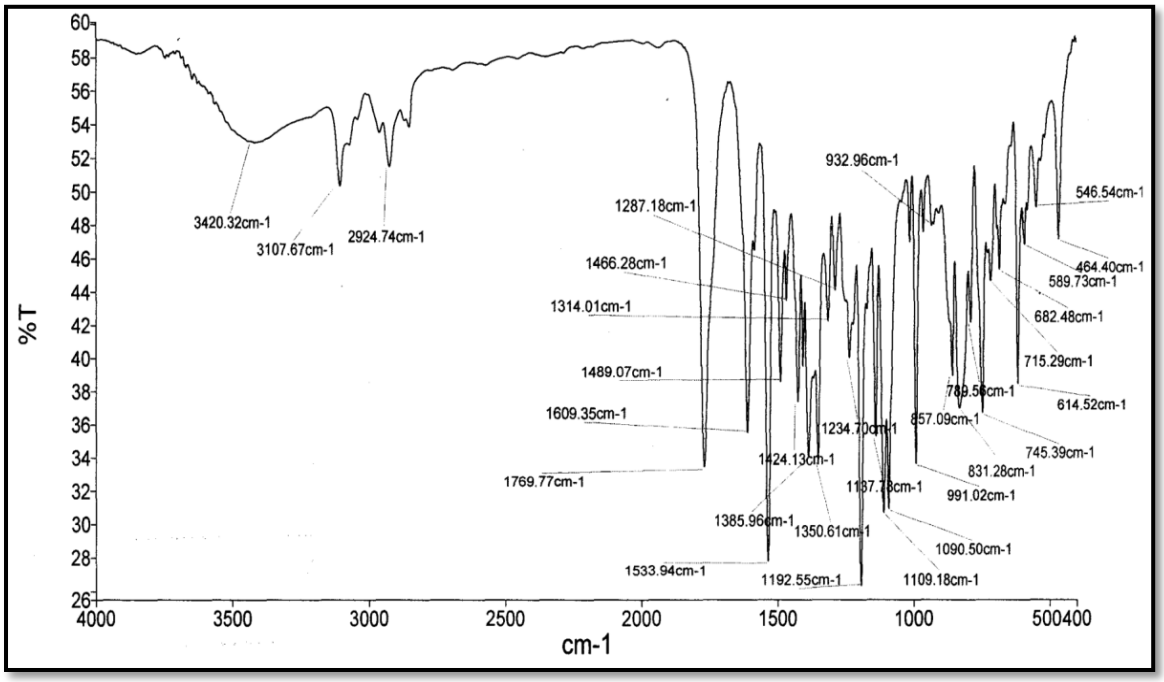


Figure S8: Mass spectrum of **FLHP**:

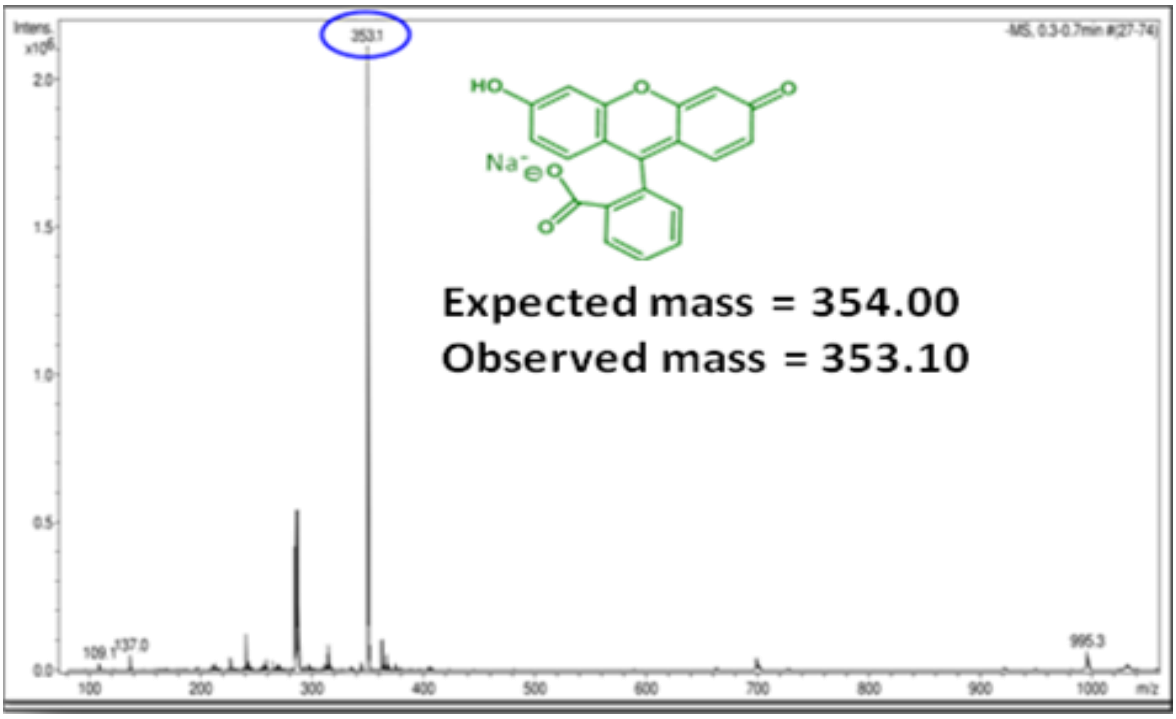


Figure S9: UV-visible spectra of **FLH** with different anions at 10μM in EtOH: water (3: 2, v/v) medium:

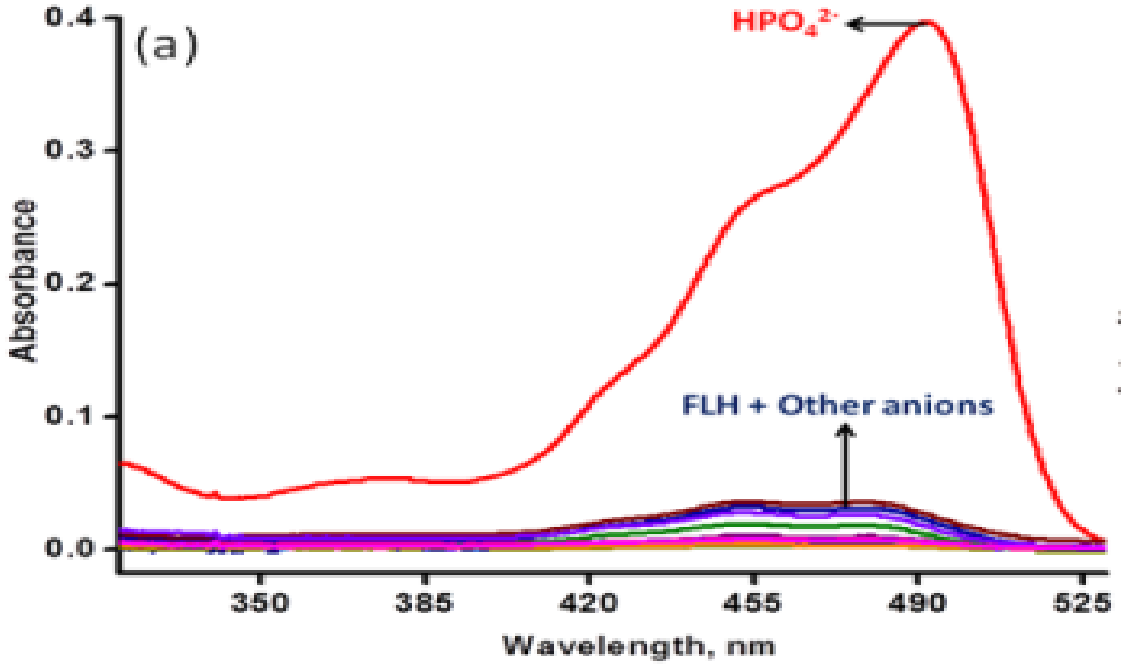


Figure S10: Bar graph representation of Absorption spectrum for competition study; **[yellow bars]** showing response **FLH** in presence of various anions, **[blue bars]** showing response of **FLH** in presence of HPO_4^{2-} and HPO_4^{2-} followed by various competing anions:

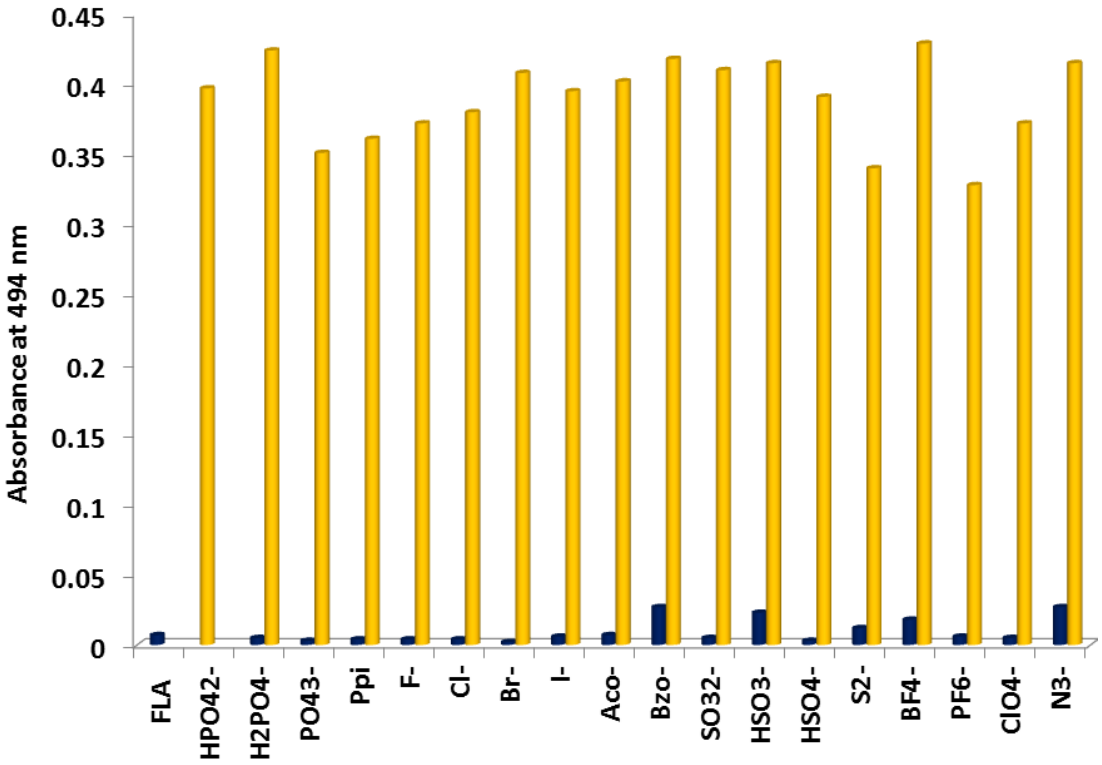


Figure S11: Naked-eye images of **FLH** in the presence of HPO_4^{2-} and various anions (under visible light):

Figure S12: Naked eye fluorescence images of **FLH** (1.0μM) in the presence of HPO_4^{2-} and various anions (10 equiv.):

Figure S13: Fluorescence spectra of receptor **FLH** (1.0µM) upon addition of different anions:

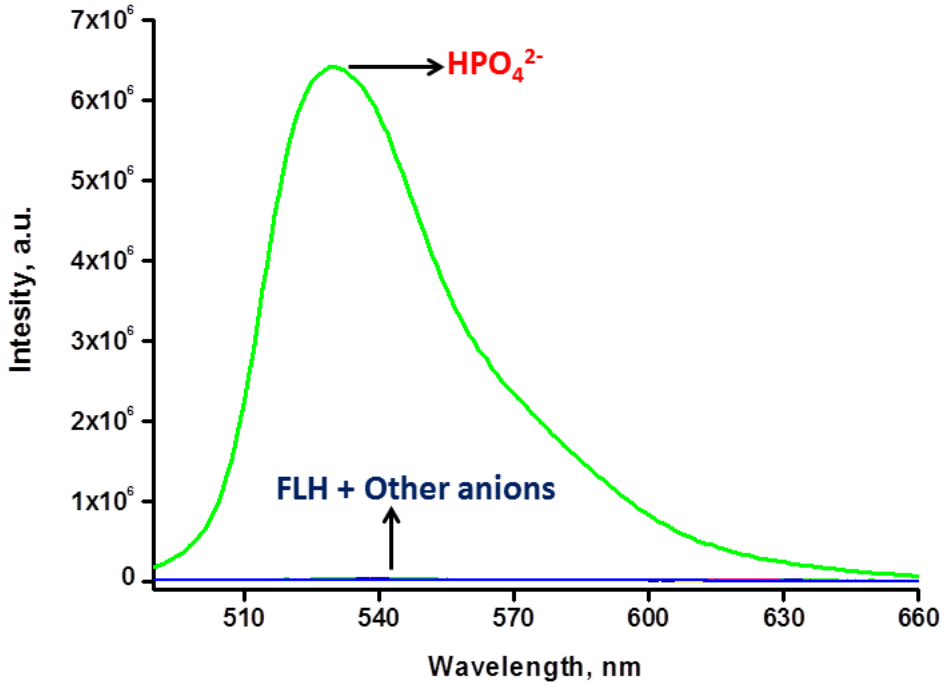


Figure S14: Bar graph representation of emission spectrum for competition study; **[green bars]** showing response **FLH** in presence of various anions, **[red bars]** showing response of **FLH** in presence of $HPO_4^{2-}$and HPO_4^{2-}followed by various competing anions:

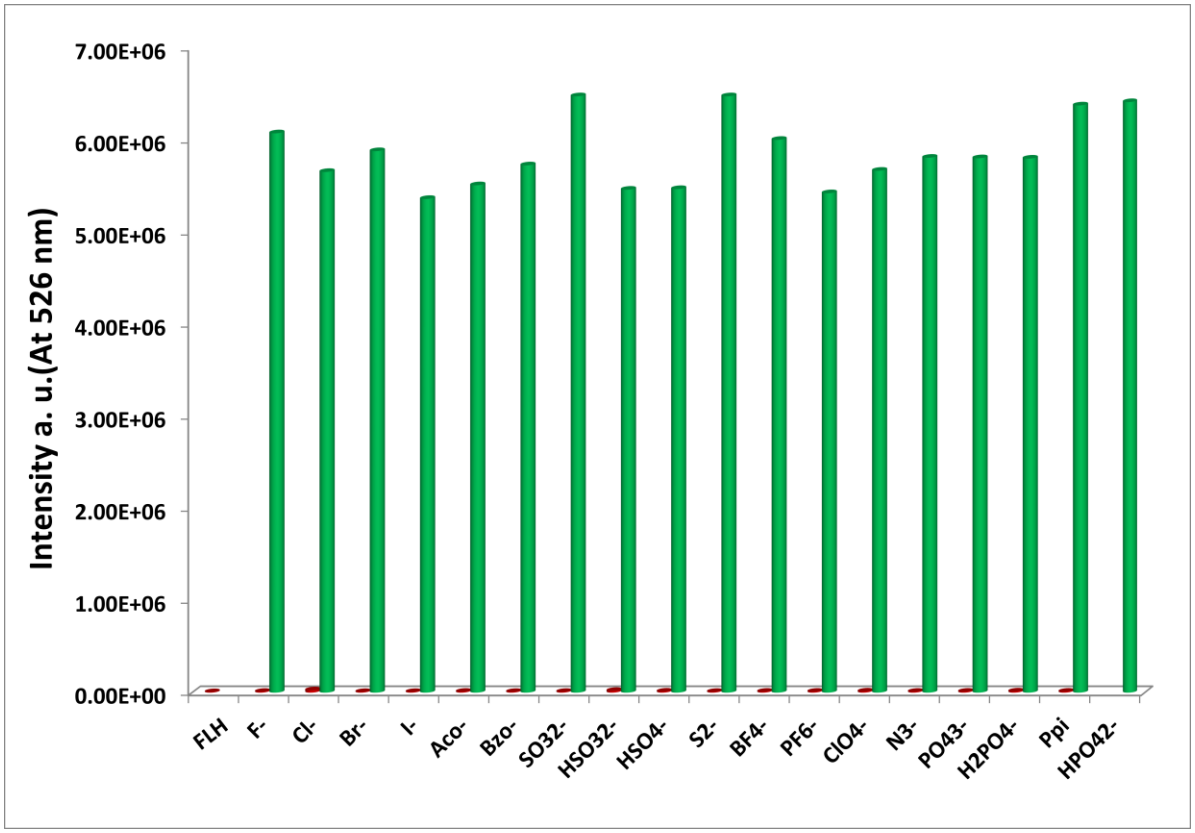


Figure S15: Calibration curve for determination of detection limit of **FLH** for HPO_4^{2-} by using absorption titration data (494 nm):

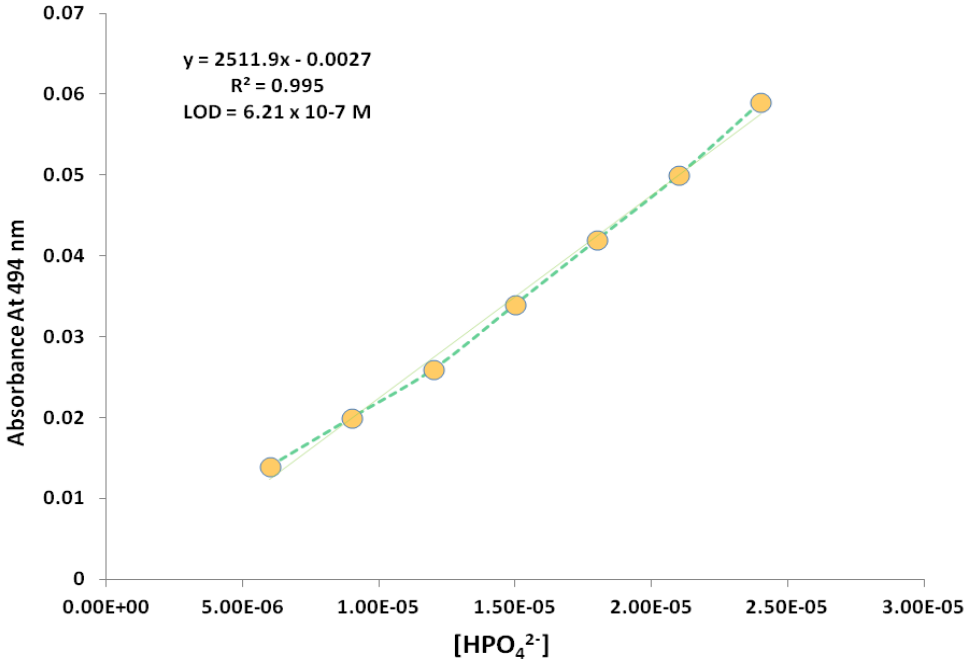


Figure S16: Calibration curve for determination of detection limit of **FLH** for HPO_4^{2-} by using emission titration data (526 nm):

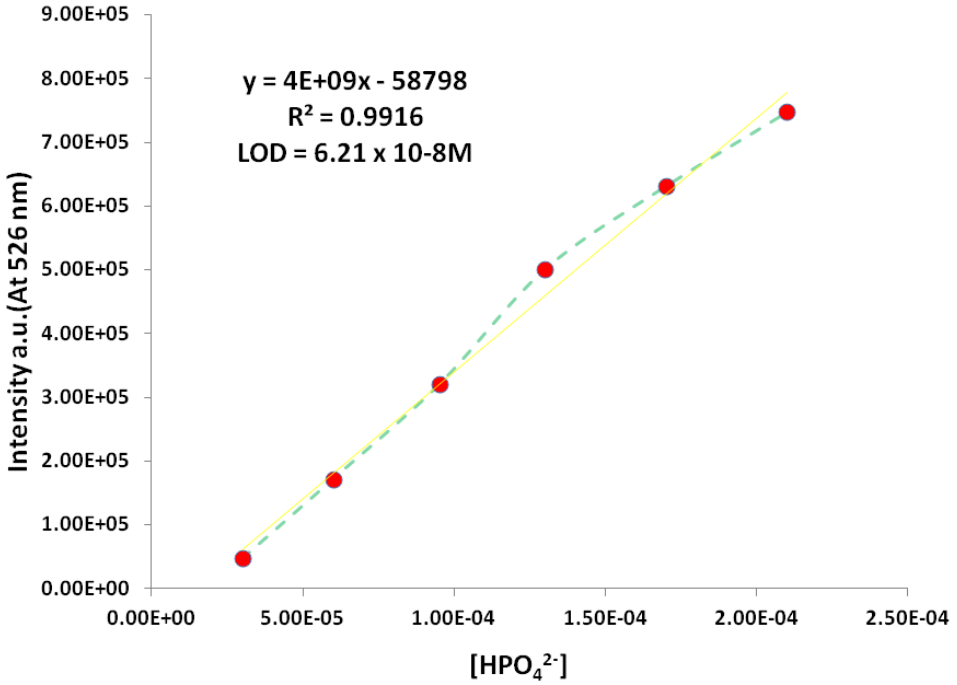


Figure S17: Fluorescence spectra of receptor **FLH** (1.0µM) upon addition different anions and corresponding fluorescence images

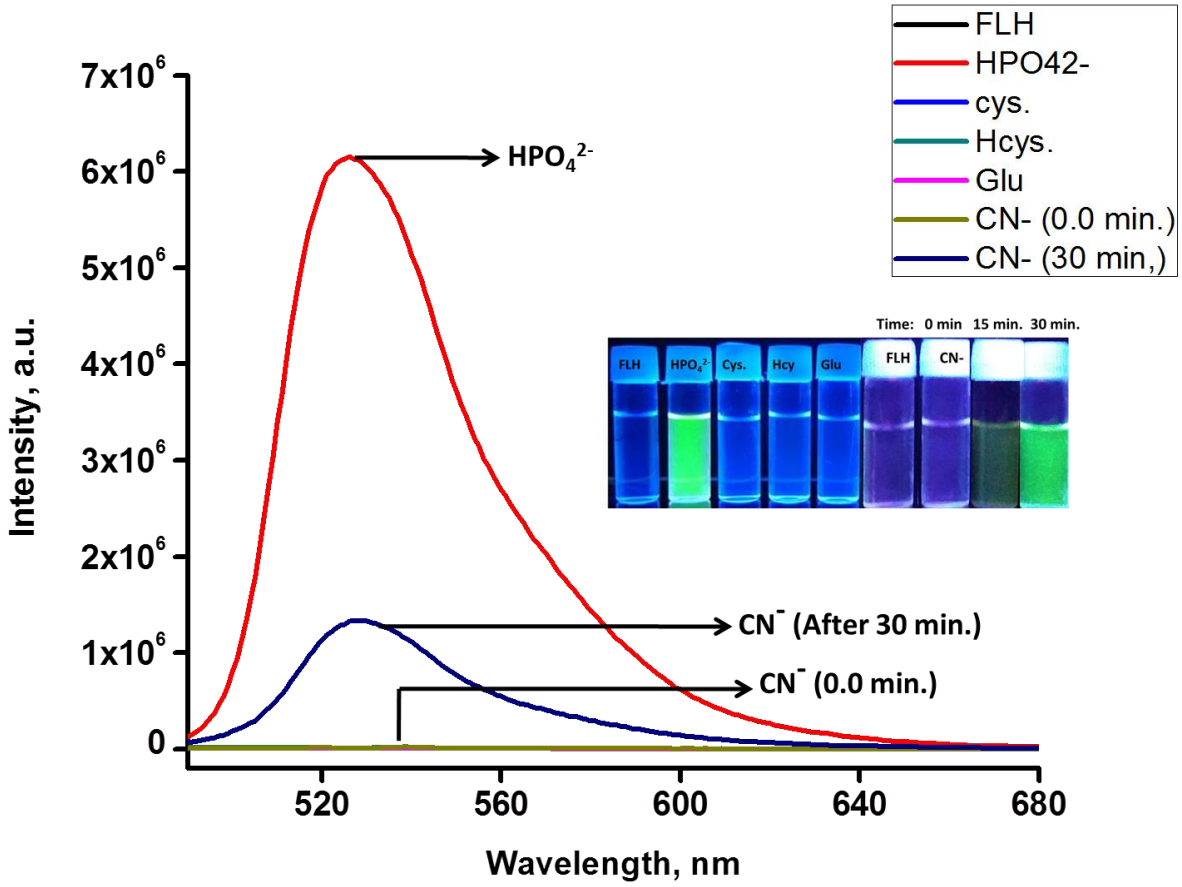
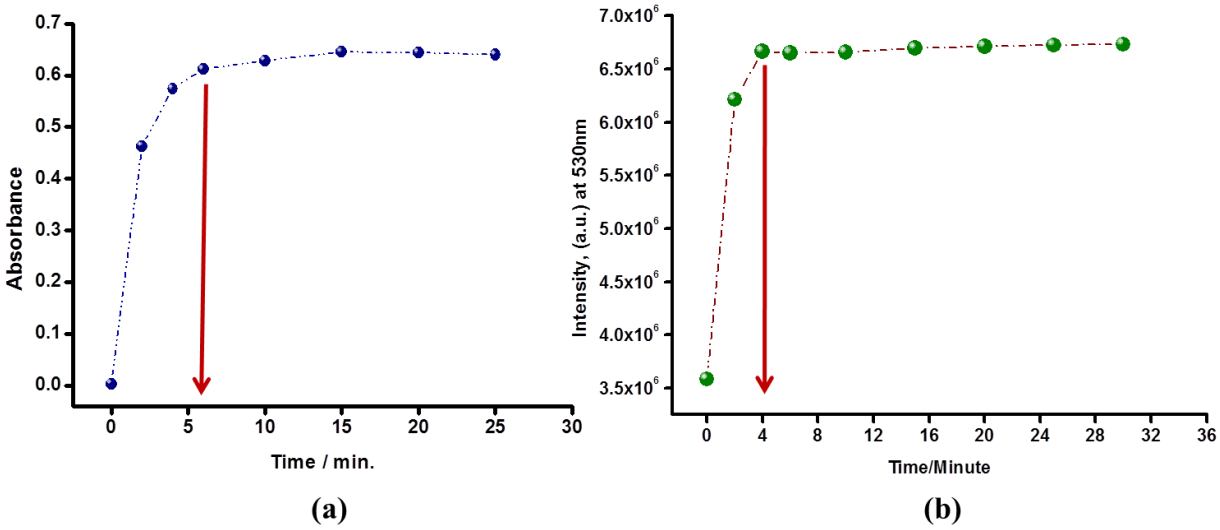


Figure S18: Reaction time profile of **FLH** with HPO_4^{2-} by **(a)** Through UV-visible spectra (λ_{abs} at 495 nm) and **(b)** Fluorescence spectra λ_{em} at 530 nm.



(a) **(b)**

Figure S19: The variation in fluorescence intensity in **FLH** with the change in pH in the presence of HPO_4^{2-}:

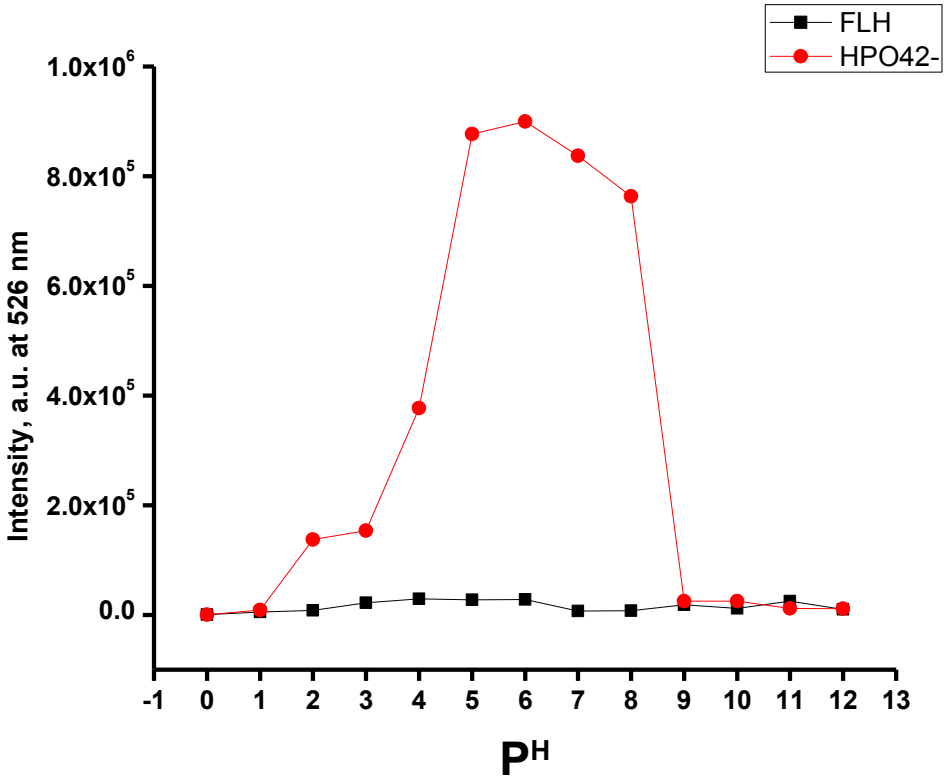


TABLE S1: Crystal data of **FLHP**

Identification code	**FLHP**
CCDC number	**994988**
Empirical formula	$C_{20}H_{14}O_6$
Formula weight	350.31
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P -1
Unit cell dimensions	a = 8.1166(11) A alpha = 70.890(10) deg. b = 9.7387(12) A beta = 69.248(11) deg. c = 11.3513(12) A gamma = 77.970(11) deg.
Volume	788.47(17)A^3
Absorption coefficient	0.110 mm^{-1}
F(000)	364.0
Crystal size	0.24 x 0.20 x 0.18 mm
Theta range for data collection	3.31 to 29.02 deg.
Limiting indices	-10<=h<=9, -11<=k<=12, -12<=l<=14
Reflections collected / unique	6075 / 3557 [R(int) = 0.0390]
Completeness to theta = 25.00	99.8 %
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3557 / 0 / 240
Goodness-of-fit on F^2	1.007
Final R indices [I>2sigma(I)]	R1 = 0.0723, wR2 = 0.1541
R indices (all data)	R1 = 0.1596, wR2 = 0.2106
Largest diff. peak and hole	0.277 and -0.285 $e.A^{-3}$