# Pyridinum-based flexible tripodal cleft: A case of fluorescence sensing of ATP and dihydrogenphosphate under different conditions and cell imaging

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## 1. Change in emission of receptor 1 with various anions of sodium salt in $CH_3CN$-$H_2O$ (1:1/v/v, using 10 mM HEPES, pH-6.5).

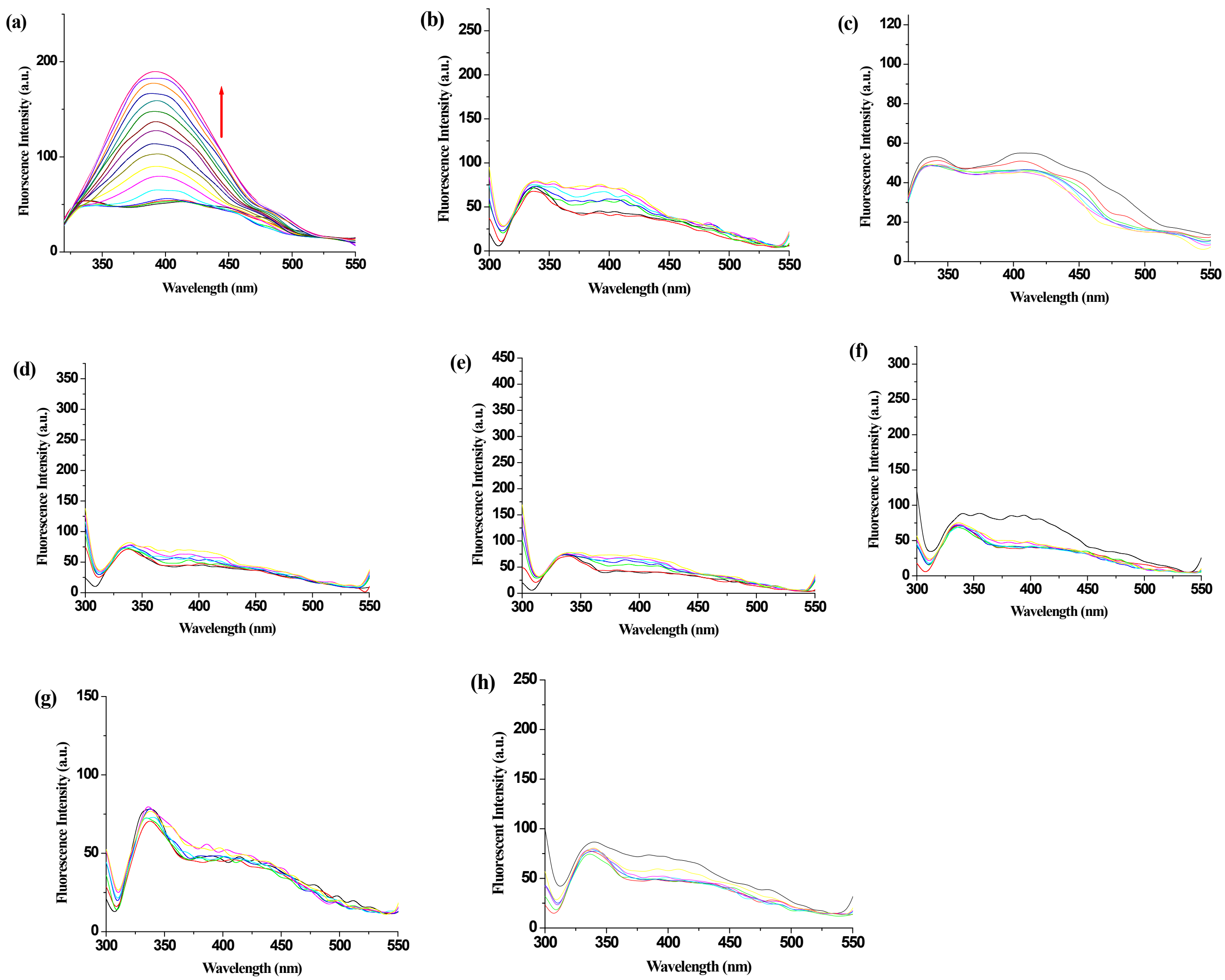



**Figure 1S**. Change in emission of **1** ($c$ = 2.5 x $10^{-5}$ M) in $CH_3CN$-$H_2O$ (1:1/v/v, using 10 mM HEPES, pH = 6.5) upon addition of 30 equiv. amounts of (a) ADP , (b) $Na_2HPO_4$, (c) AMP, (d) $Na_3PO_4$, (e) $NaH_2PO_4$, (f) $Na_4P_2O_7$, (g) glucose-1- phosphate (G1P), (h) glucose-6-phosphate (G6P) [concentration of anions of sodium salts were 1 x $10^{-3}$ M].

## 2. Interference Study in the binding of ATP

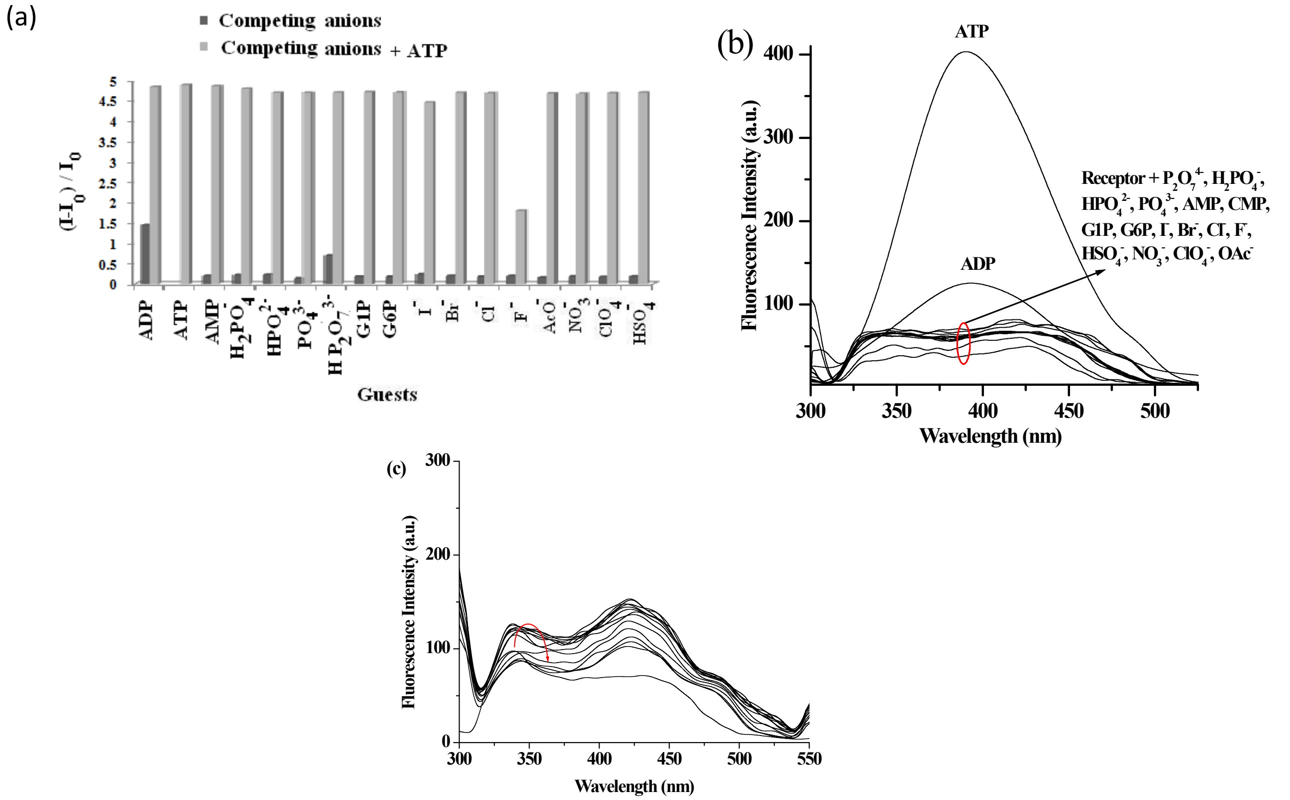



**Figure 2S.** (a) Change in fluorescence ratio of **1** (c = 2.5 x $10^{-5}$ M) in absence and presence of 20 equiv. amounts of ATP in presence of sodium salts of various anions in $CH_3CN–H_2O$ (1: 1, v/v, pH = 6.5, 10 mM HEPES buffer); (b) change in fluorescence intensity of **1** in $CH_3CN–H_2O$ (1: 1, v/v, pH = 6.5, 10 mM HEPES buffer) upon addition of 20 equiv. amounts of ATP in presence of other anions; (c) Fluorescence titration spectra of **1** (c = 2.5 x $10^{-5}$ M) with tetrabutylammonium fluoride ($c$ = 1.0 x $10^{-3}$ M).

## 3. Benesi-Hilderband plot for receptor 1 with ADP.

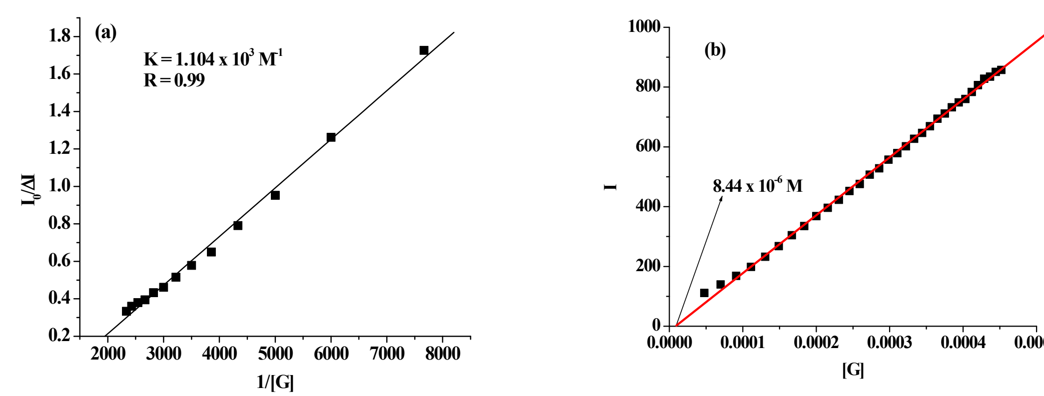



**Figure 3S.** (a) Benesi-Hilderband plot for receptor **1** (c = 2.5 x $10^{-5}$ M) with ADP ([ADP] = 1 x $10^{-3}$ M) at 390 nm; (b) Detection limit for ATP in $CH_3CN$-$H_2O$ (1:1/v/v, using 10 mM HEPES, pH = 6.5).

## 4. Change in absorbance of receptor 1 with various anions of sodium salt in $CH_3CN$-$H_2O$ (1:1/v/v, using 10 mM HEPES, pH = 6.5).

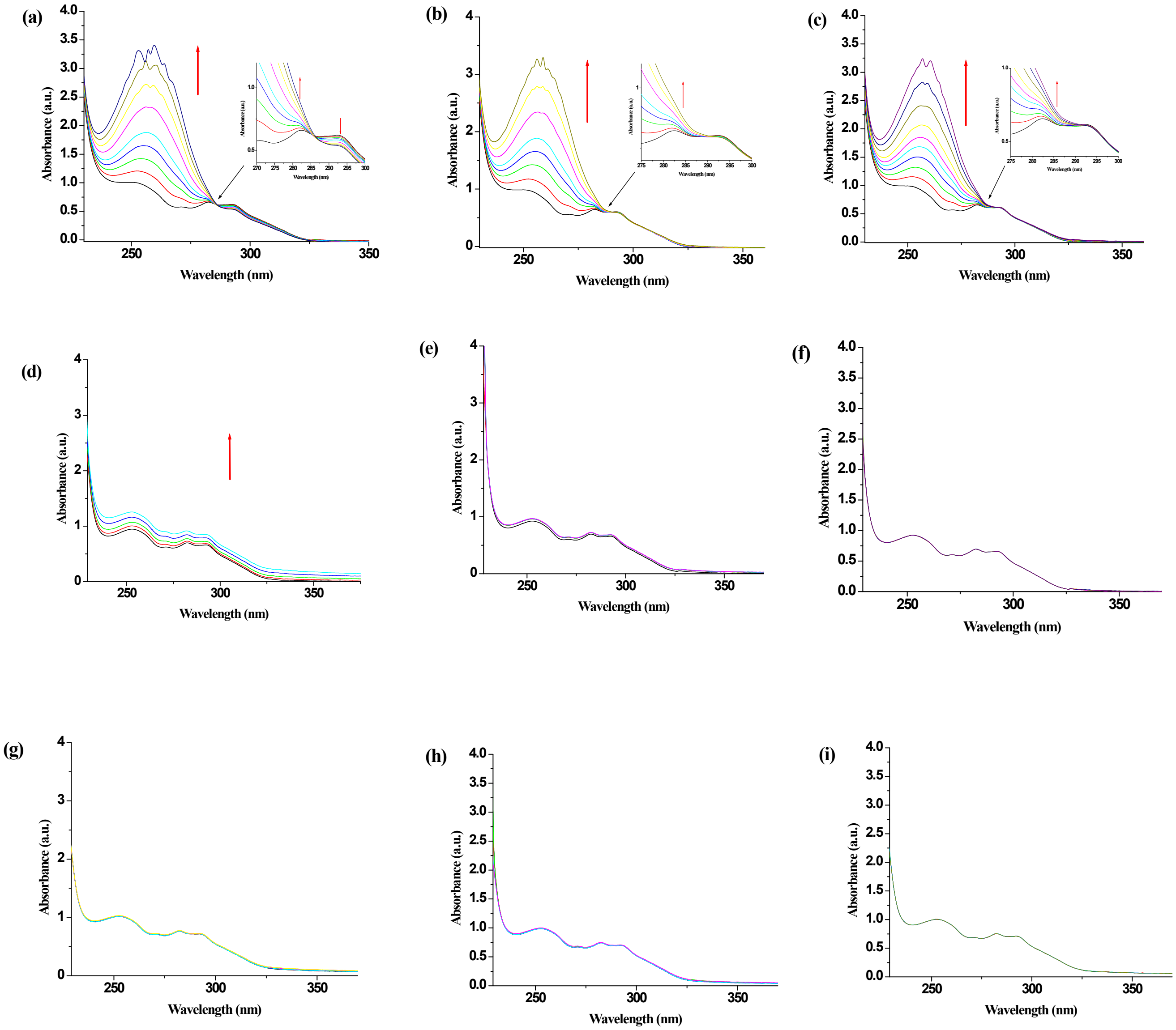



**Figure 4S.** Change in absorbance of **1** ($c$ = 2.5 x $10^{-5}$ M) in $CH_3CN$-$H_2O$ (1:1/v/v, using 10 mM HEPES, pH-6.5) upon addition of 10 equiv. amounts of (a) ATP , (b) ADP, (c) AMP, (d) $NaH_2PO_4$, (e) $Na_2HPO_4$, (f) $Na_3PO_4$, (g) $Na_4P_2O_7$, (h) G1P, (i) G6P [concentration of anions of sodium salts were 1 x $10^{-3}$ M].

## 5. Change in emission of receptor 1 with various anions of tetrabutylammonium salts in $CH_3CN$.

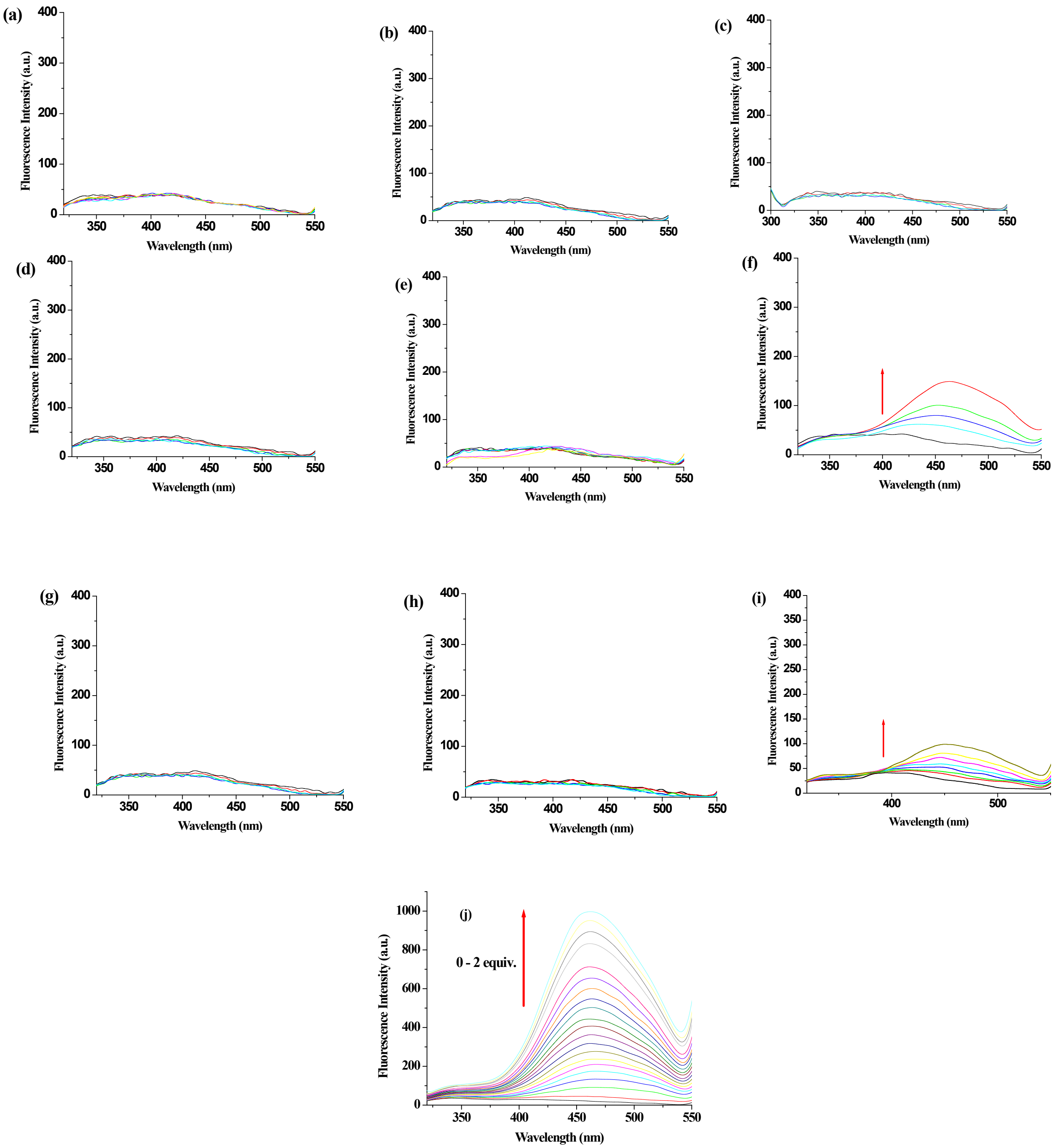



**Figure 5S**. Change in emission of **1** ($c$ = 2.2 x $10^{-5}$ M) in $CH_3CN$ upon addition of 2 equiv. amounts of (a) $OAc^-$, (b) $Cl^-$ (c) $Br^-$, (d) $I^-$ , (e) $F^-$, (f) $HP_2O_7^{3-}$, (g) $NO_3^-$, (h) $ClO_4^-$ , (i) $HSO_4^-$ and (j) $H_2PO_4^-$ [anions were taken as their tetrabutylammonium salts and their concentrations were 8 x $10^{-4}$ M].

## 6. Interference study towards binding of $H_2PO_4^-$ ion in $CH_3CN$

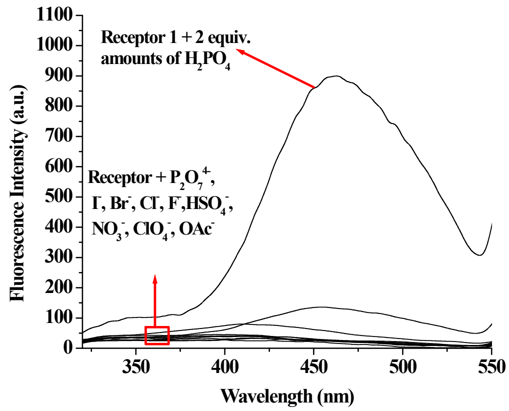
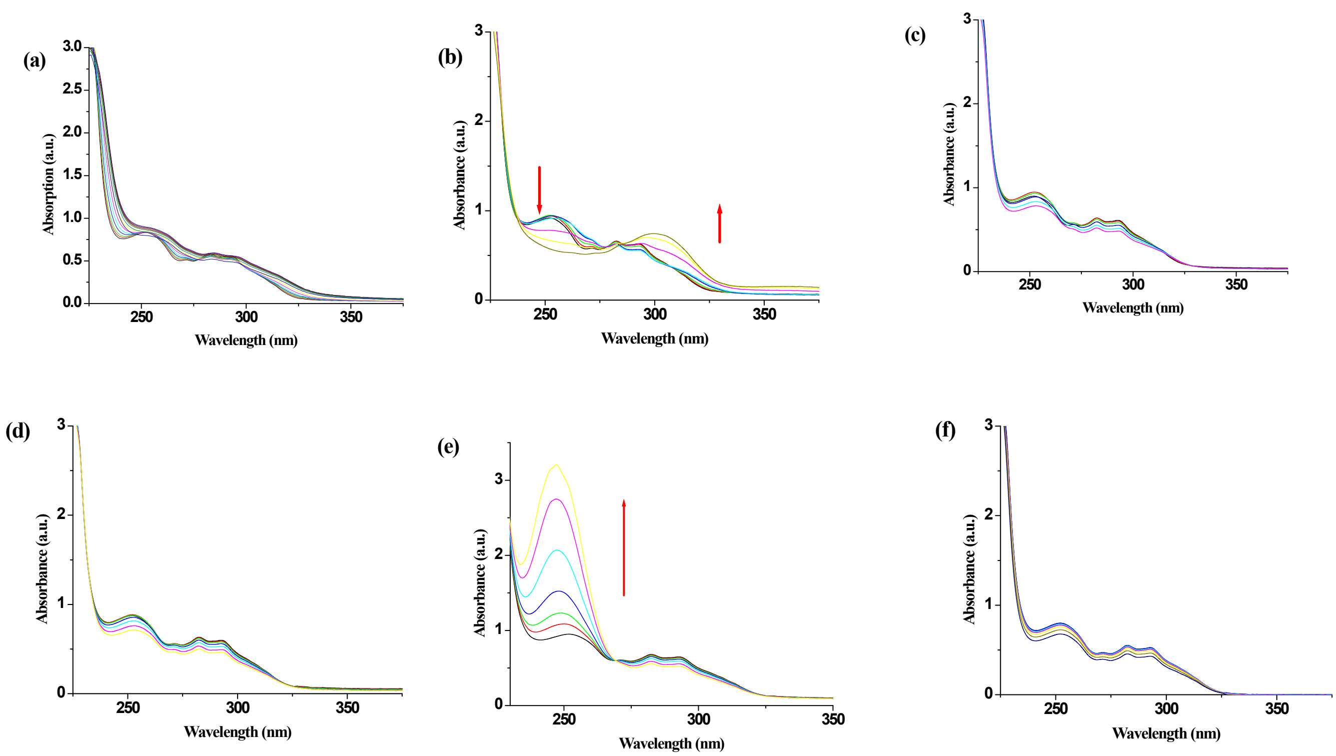



**Figure 6S**. Change in emission of **1** ($c$ = 2.2 x $10^{-5}$ M) upon addition of 2 equiv. amounts of tetrabutylammonium dihydrogenphosphate (TBADHP) ($c$ = 8 x $10^{-4}$ M) in presence of 2 equiv. amounts of other anions as their tetrabutylammonium salts.

## 7. Change in absorbance of receptor 1 with various anions of tetrabutylammonium salts in $CH_3CN$.



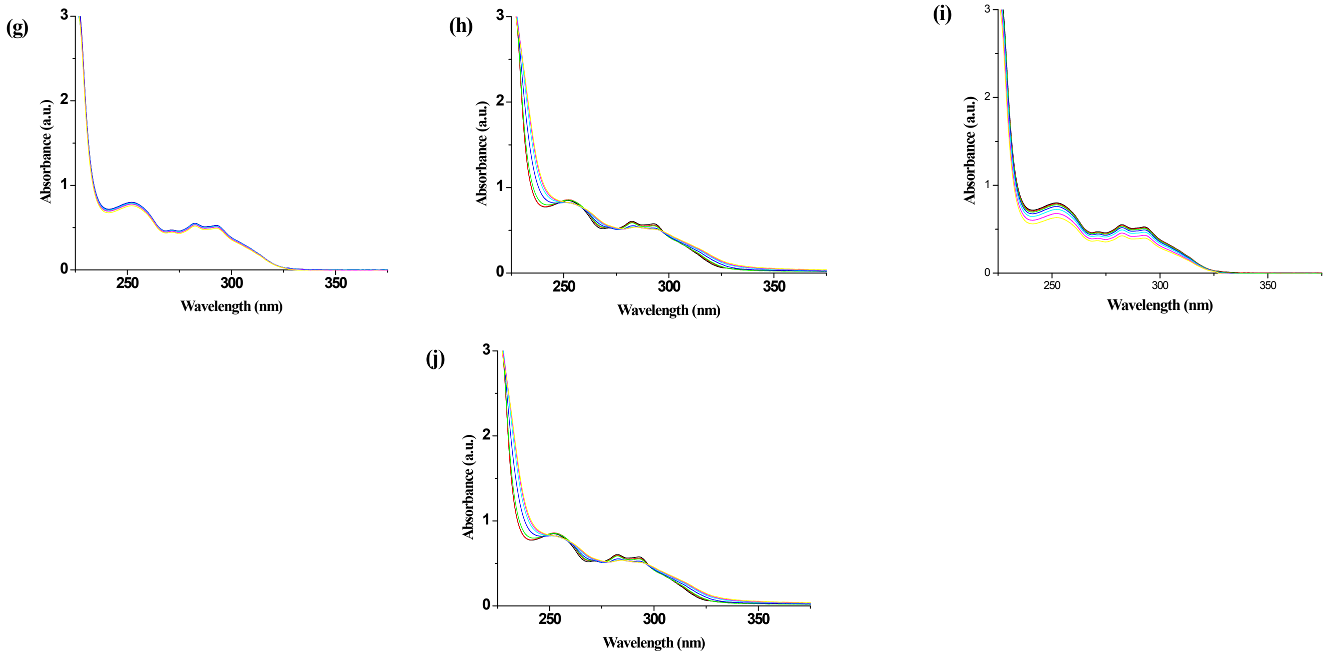



**Figure 7S**. Change in absorbance of **1** ($c$ = 2.2 x $10^{-5}$ M) in $CH_3CN$ upon addition of 10 equiv. amounts of (a) $H_2PO_4^-$, (b) $F^-$, (c) $Cl^-$, (d) $Br^-$, (e) $I^-$, (f) $OAc^-$, (g) $HP_2O_7^{3-}$, (h) $NO_3^-$, (i) $ClO_4^-$, (j) $HSO_4^-$ [anions were taken as their tetrabutylammonium salts and their concentrations were 8 x $10^{-4}$ M].

## 8. Fluorescence Job plot of receptor 1 with $H_2PO_4^-$ in $CH_3CN$.

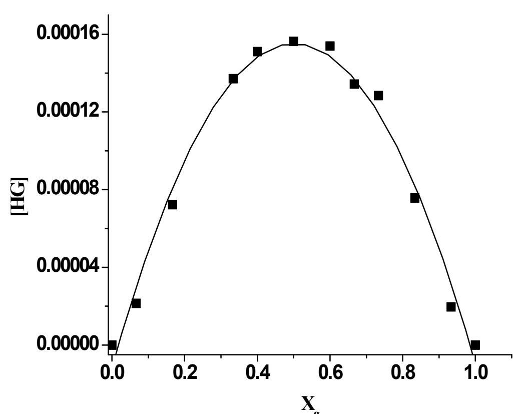



**Figure 8S**. Fluorescence Job's plot for receptor **1** with $H_2PO_4^-$ in $CH_3CN$ measured at 455 nm ([H] = [G] = 2.5 x $10^{-5}$ M).

## 9. Benesi-Hilderband plot for receptor 1 with $H_2PO_4^-$.

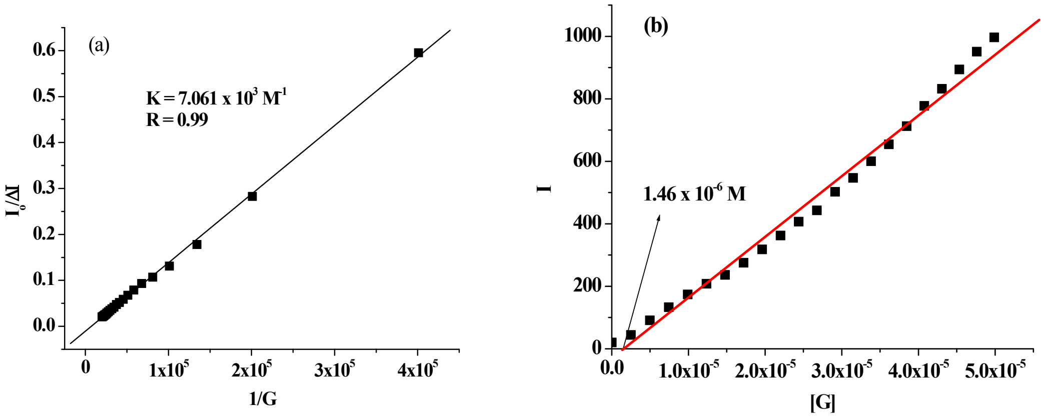



**Figure 9S**. (a) Binding constant curve for receptor **1** ($c$ = 2.2 x $10^{-5}$ M) with $H_2PO_4^-$ ($c$ = 8 x $10^{-4}$ M); (b) Detection limit for $H_2PO_4^-$ in $CH_3CN$.

## 10. NOESY spectrum

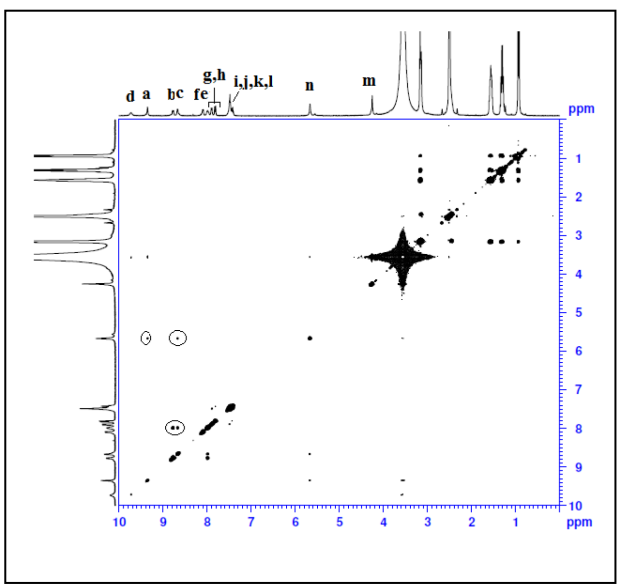
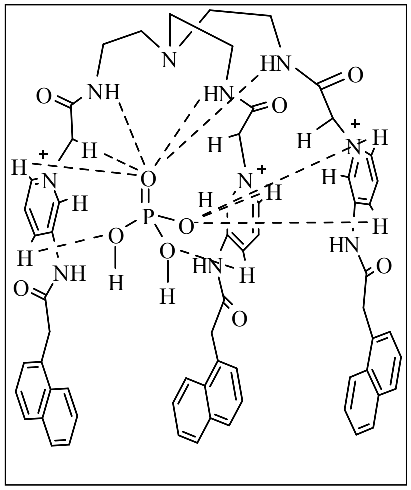



**Figure 10S.** NOESY spectrum of **1.**$H_2PO_4^-$ ($d_6$-DMSO/$D_2O$, 400 MHz) (c = 1.83 x $10^{-3}$ M).

## 11. Change in $^1$H NMR of 1 in presence of $H_2PO_4^-$.



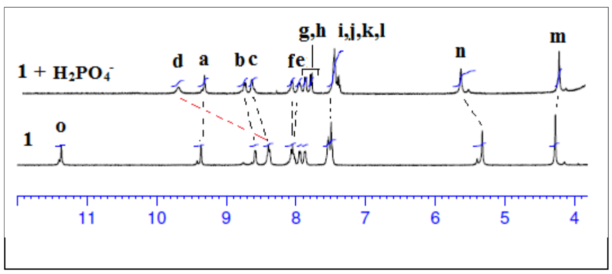



**Figure 11S.** Partial $^1$H NMR ($d_6$-DMSO, 400 MHz) of **1** (c = 1.83 x $10^{-3}$ M) with equiv. amount of TBADHP. The mode of interaction is shown above. Dash lines indicate the possible weak H-bond interactions or short contacts in the core in a symmetric fashion.

## 12. MTT assay for receptor 1.

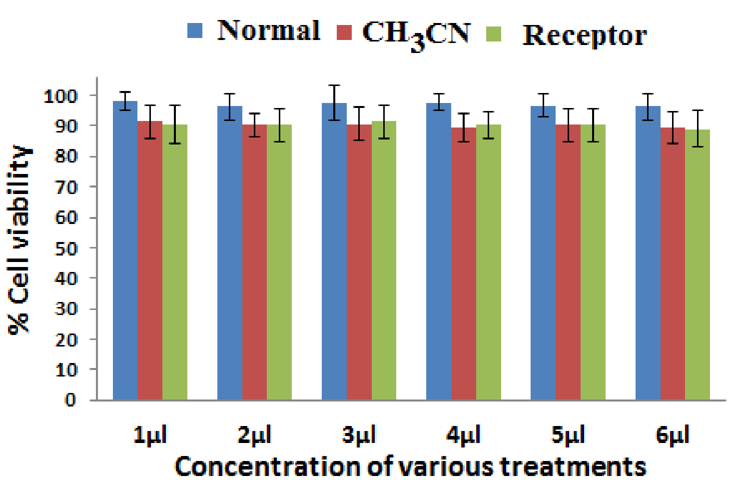
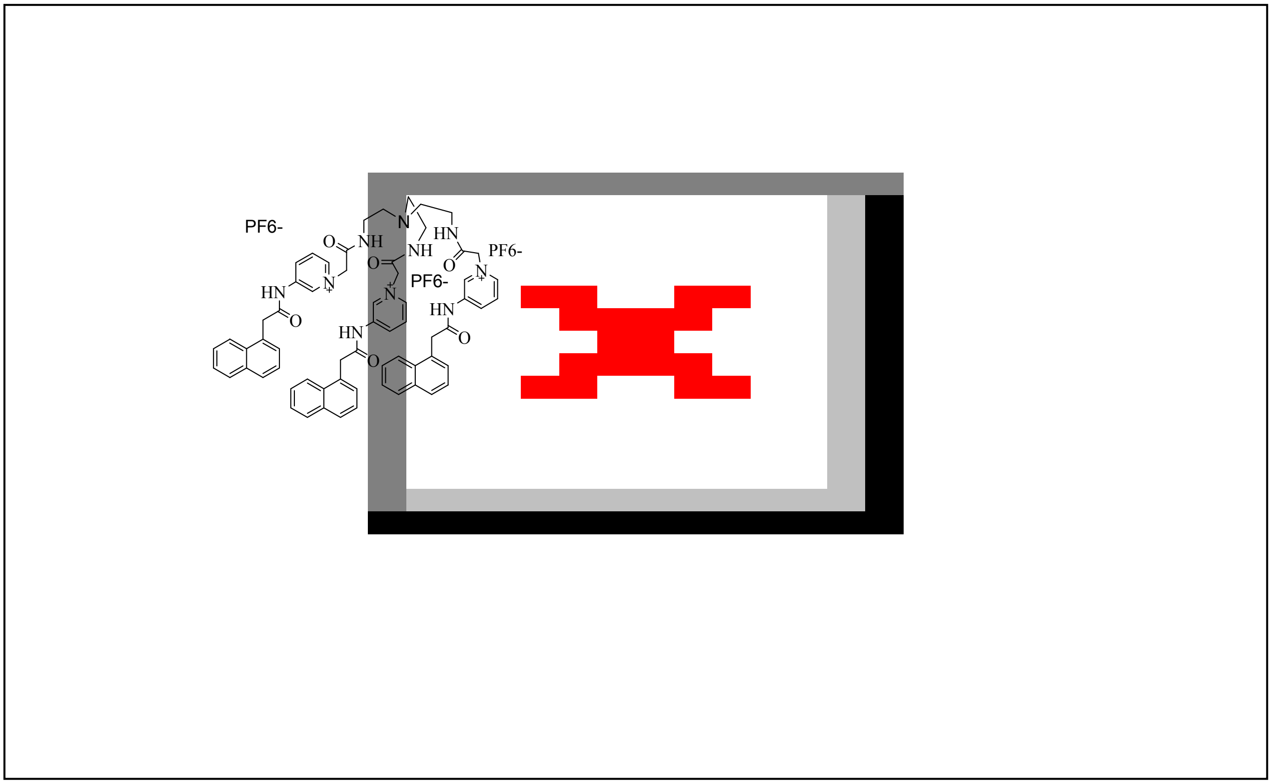
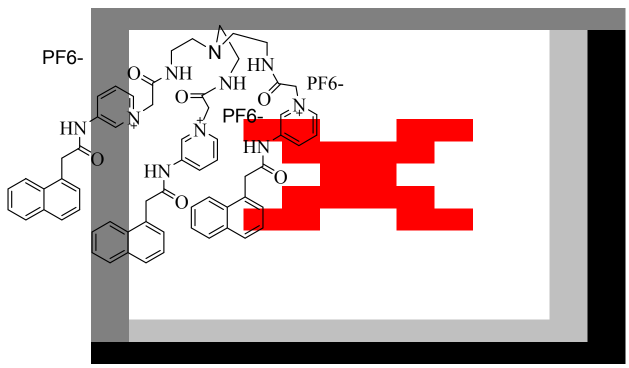



**Figure 12S**. MTT assay of receptor **1**.

**$^{1}$H NMR of 1 (400 MHz, $d_6$-DMSO):**



**$^{13}$C NMR of 1 (100 MHz, $d_6$-DMSO ):**



**HRMS OF 1:**