Supporting Information

Pyridinium-based tripodal chemosensor in visual sensing of AMP in water by indicator displacement assay (IDA)

Kumaresh Ghosh[*],[a] Sk. Sarfaraj Ali,[a] Avik Ranjan Sarkar,[a] Asmita Samadder[b], Anisur Rahman Khuda-Bukhsh,[b] Ioannis D. Petsalakis[c] and Giannoula Theodorakopoulos[c]

[a]*Department of Chemistry, University of Kalyani, Kalyani-741235, India. Email: ghosh_k2003@yahoo.co.in, Fax: +913325828282; Tel: +913325828750.*
[b]*Department of Zoology, University of Kalyani, Kalyani-741235, India.*
[c] *Theoretical and Physical Chemistry Institute, The National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, Athens 11635, Greece.*

1. UV-vis titration curve of receptor 1 in H_2O (pH = 6.4, 10 mM TrisHCl Buffer)

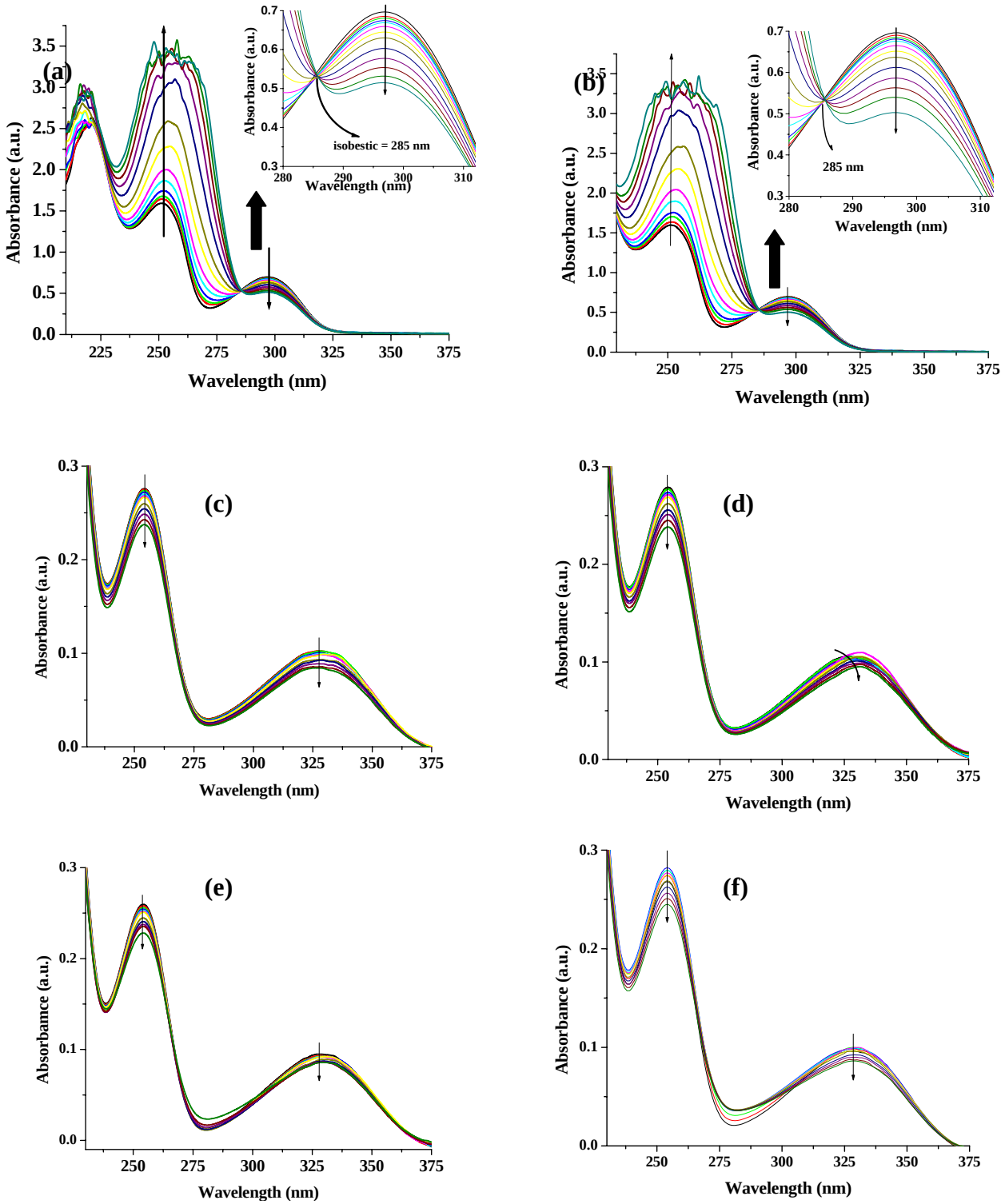


Figure S1: UV-vis titration curves of **1** (c = 6.7 x 10^{-5} M) with Na salt of (a) ATP, (b) ADP, (c) $H_2PO_4^-$, (d) HPO_4^{2-}, (e) PO_4^{3-} and (f) pyrophosphate in H_2O (pH = 6.4, 10 mM TrisHCl Buffer). [G] = 1.35 x 10^{-3} M.

2. Job plots for 1 with ATP, ADP and AMP

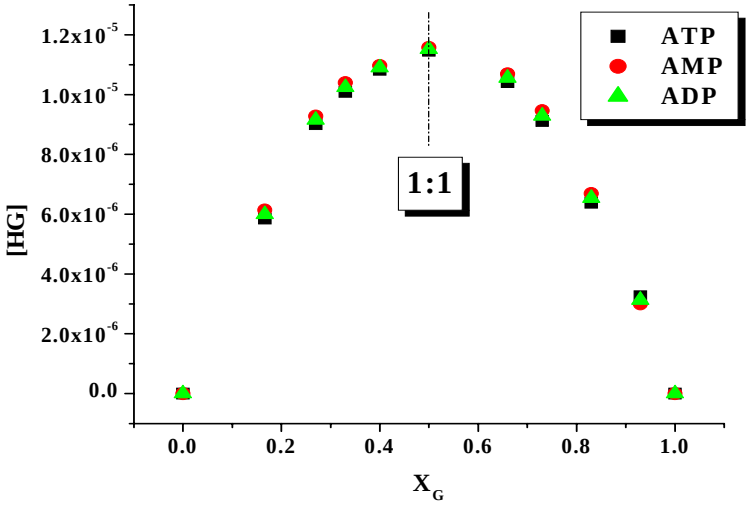
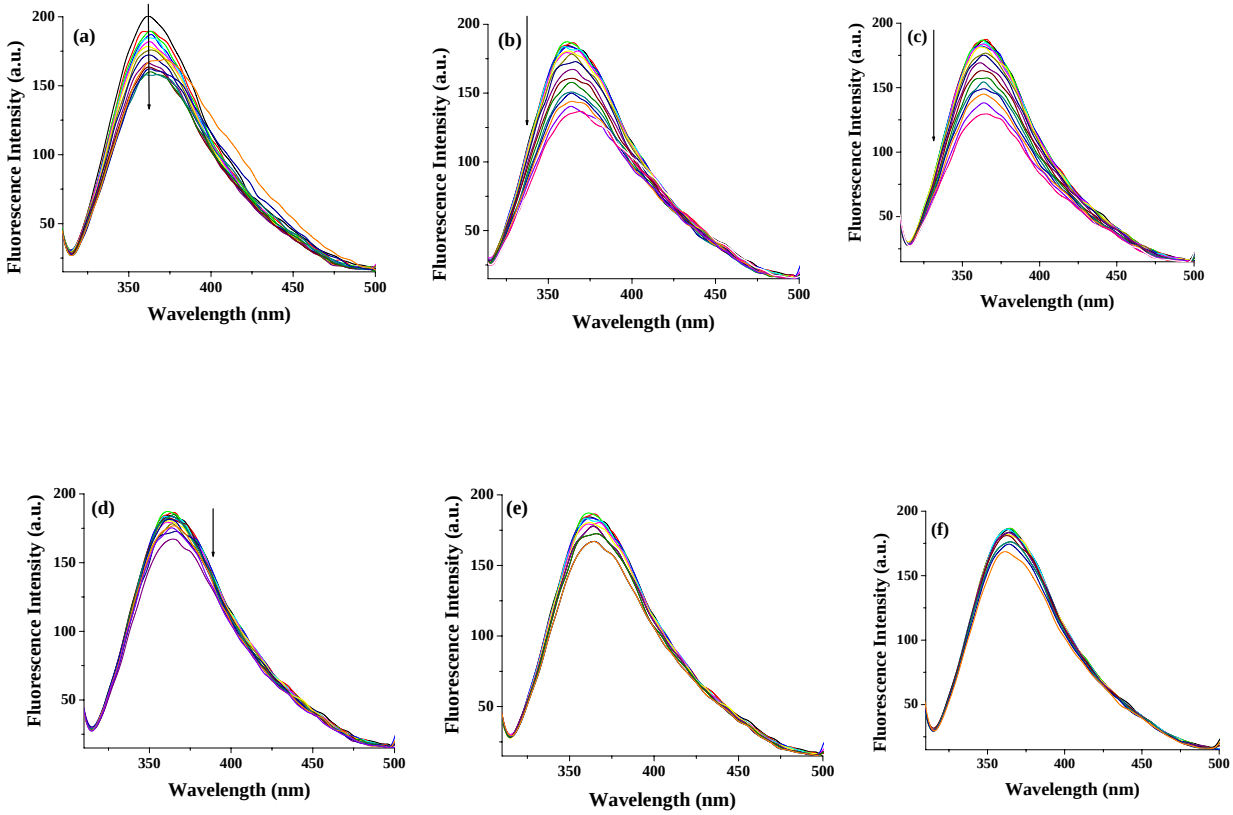


Figure S2. UV-vis job plots of **1** with (a) ATP, (b) AMP and (c) ADP at 295 nm in H_2O containing TrisHCl buffer pH= 6.4. { [G] = [H] = 5.0 x 10^{-5} M at 25 ^{o}C}.

3. Fluorescence titration spectra for 1 in H_2O (pH = 6.4, 10 mM TrisHCl Buffer)



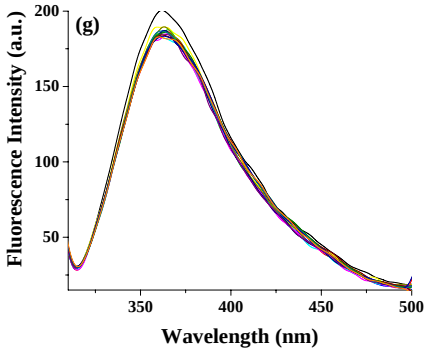


Figure S3: Fluorescence titration curves of **1** (c = 6.7 x 10^{-5} M) with Na salt of (a) ATP, (b) ADP, (c) AMP, (d) $H_2PO_4^-$, (e) HPO_4^{2-}, (f) PO_4^{3-} and (g) pyrophosphate in H_2O (pH = 6.43, 10 mM TrisHCl Buffer). [G] = 1.35 x 10^{-3} M.

4. Fluorescence titration spectra for 1 with AMP in H_2O (pH = 6.4, 10 mM TrisHCl Buffer)

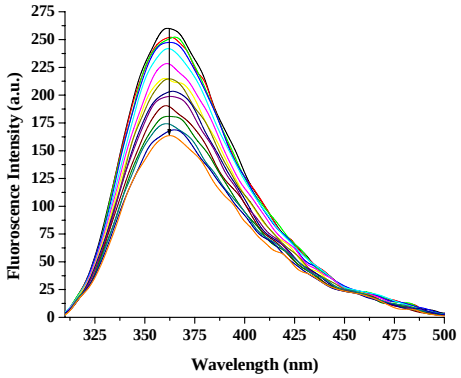


Figure S4. Fluorescence titration spectra of **1**with AMP on excitation at 285 nm ([H] = 6.75 x 10^{-5} M, [G] = 1.35 x 10^{-3} M).

5. Dilution Experiments of receptor 1 in H_2O containing TrisHCl buffer pH= 6.4

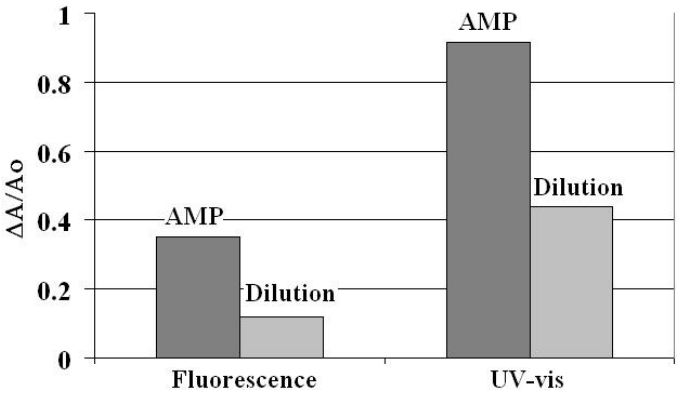


Figure S5. Change in emission and absorption intensity due to addition of AMP and solvent (H_2O) to the solution of receptor **1** in H_2O containing TrisHCl buffer pH= 6.4.

6. DFT calculation

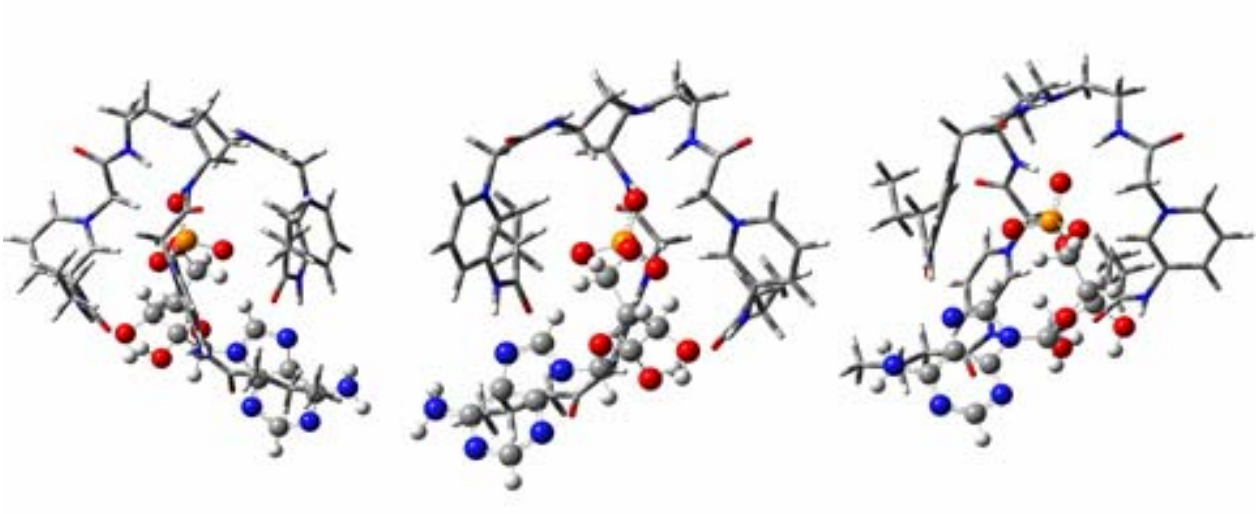


Figure S6: Three views of the optimum geometry of the complex formed by receptor **1** (shown as sticks) with AMP.

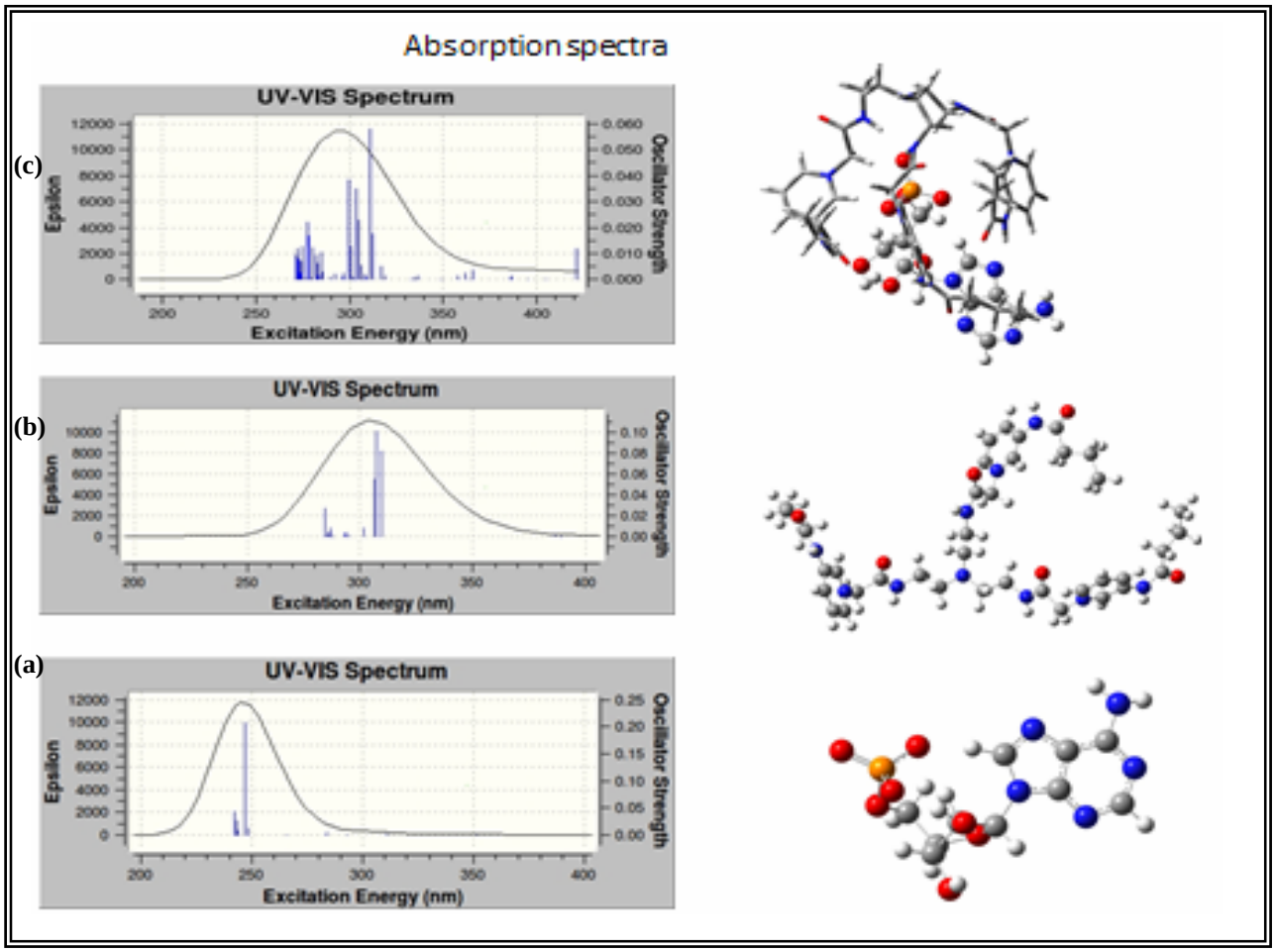


Figure S7: Lower energy absorption spectra of (a) AMP, (b) receptor **1** and (c) the complex of receptor **1** (shown as sticks) with AMP.

7. Indicator displacement experiments on 1 with Uranine dye (2):

a) UV-vis experiment

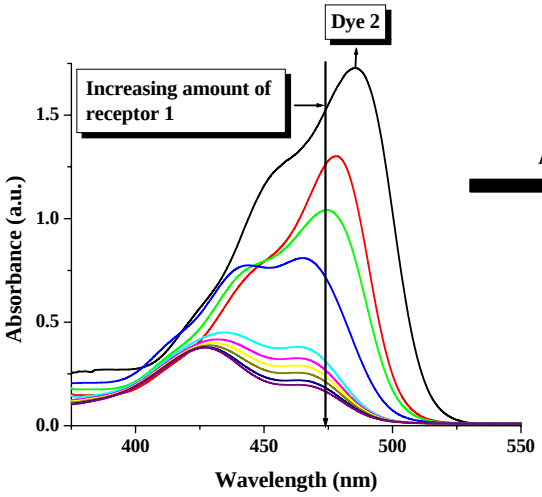


Figure S8: Addition of **1** (c = 1.6 x 10^{-3} M) into the solution of **2** (c = 4.2 x 10^{-5} M) causes a decrease in the absorption intensity of **2** at 485 nm in H_2O (pH = 6.4, 10 mM Tris/HCl buffer) at 25 ^{0}C.

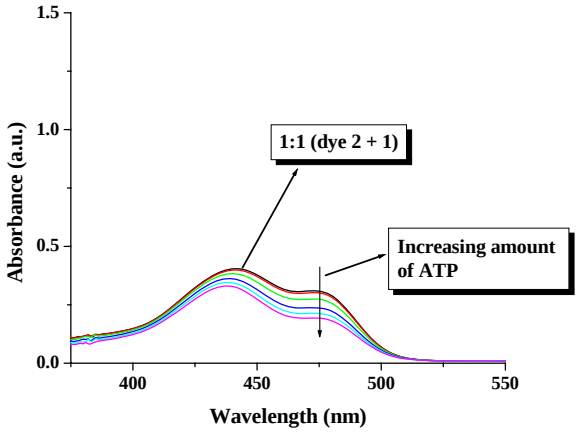


Figure S9. Change in absorbance due to gradual addition of ATP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

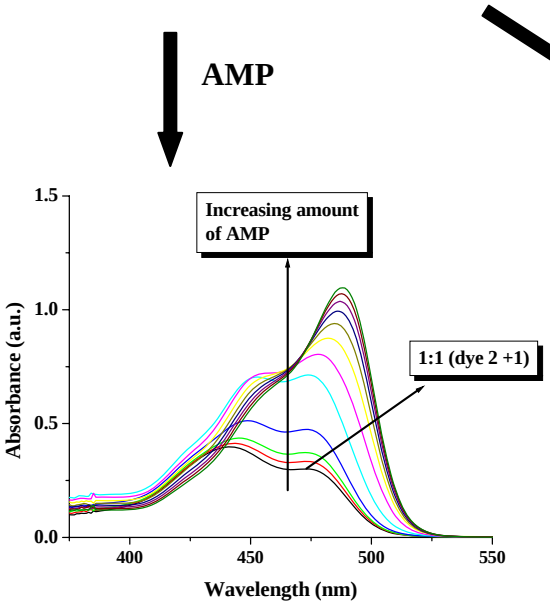


Figure S10: Change in absorbance due to gradual addition of AMP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

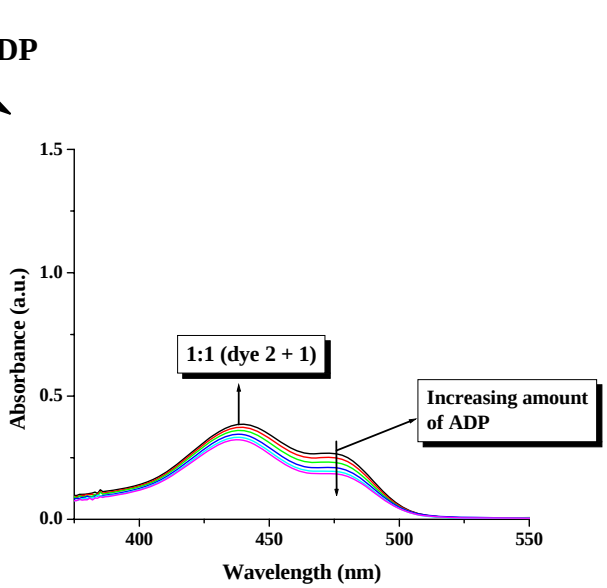


Figure S11. Change in absorbance due to gradual addition of ADP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

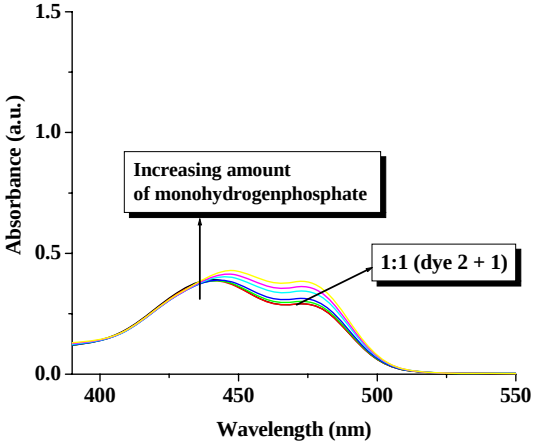


Figure S12: Change in absorbance due to gradual addition of monohydrogenphosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

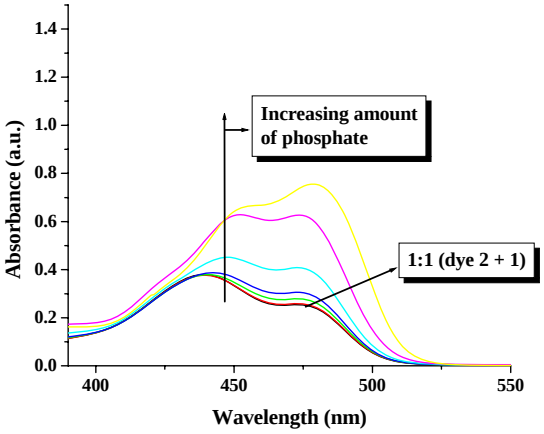


Figure S13: Change in absorbance due to gradual addition of phosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

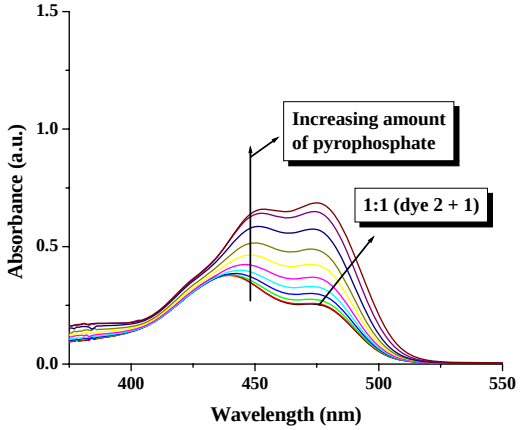


Figure S14: Change in absorbance due to gradual addition of pyrophosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

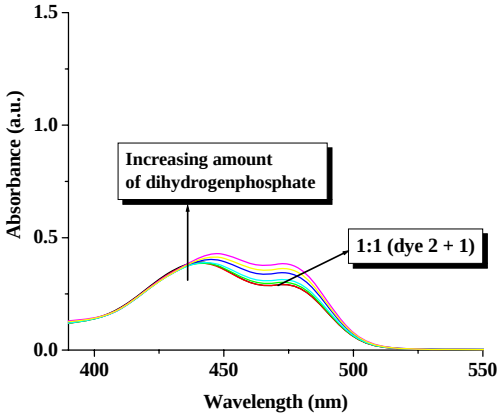


Figure S15. Change in absorbance due to gradual addition of dihydrogenphosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

b) Fluorescence experiments

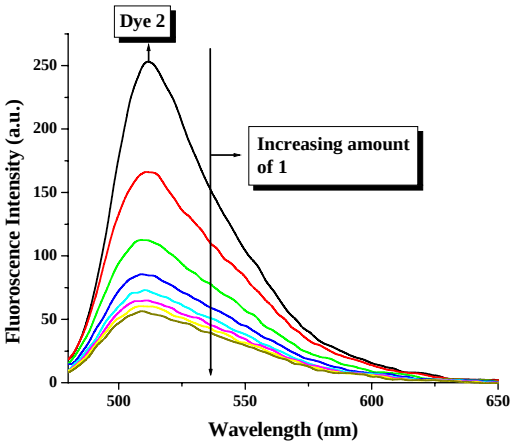


Figure S16: Addition of **1** (c = 1.6 x 10^{-3} M) into the solution of **2** (c = 4.2 x 10^{-5} M) causes a decrease in the fluorescence intensity of **2** at 511 nm in H_2O (pH = 6.4, 10 mM Tris/HCl buffer) at 25 ^{0}C.

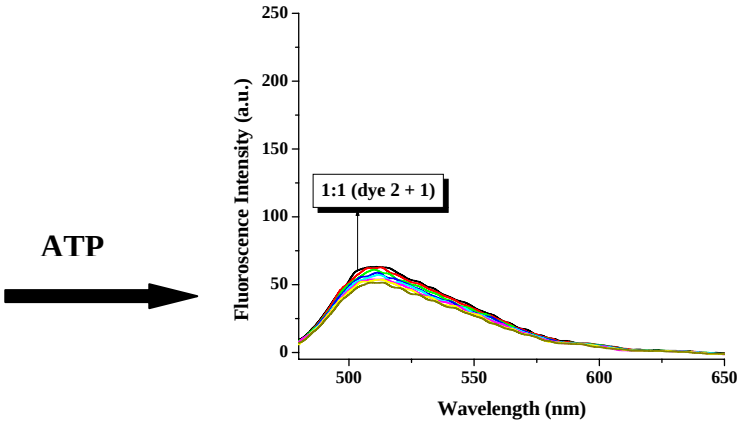


Figure S17: Change in fluorescence intensity due to gradual addition of ATP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{o}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

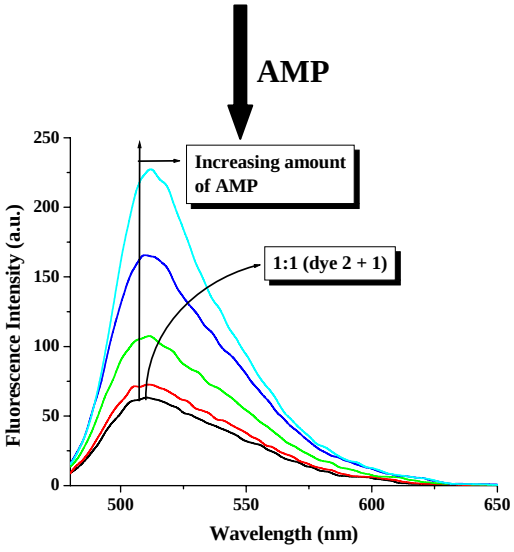


Figure S18: Change in fluorescence intensity due to gradual addition of AMP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

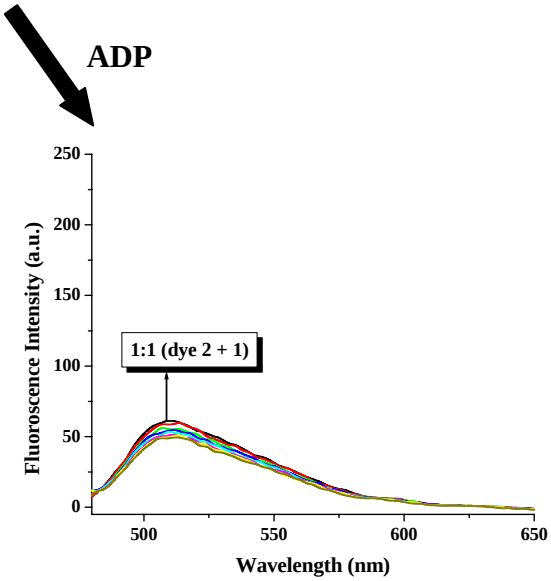


Figure S 19: Change in fluorescence intensity due to gradual addition of ADP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

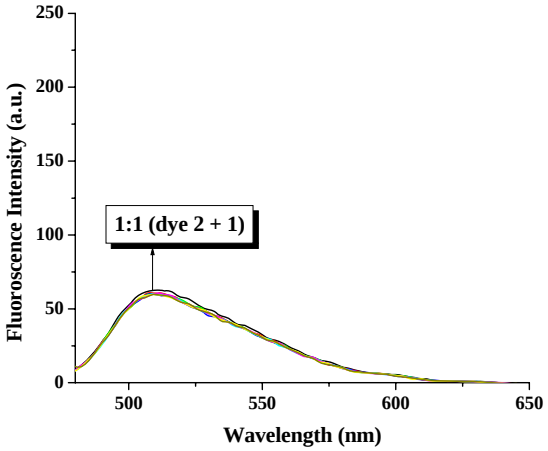


Figure S20: Change in fluorescence intensity due to gradual addition of Sodium dihydrogenphosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

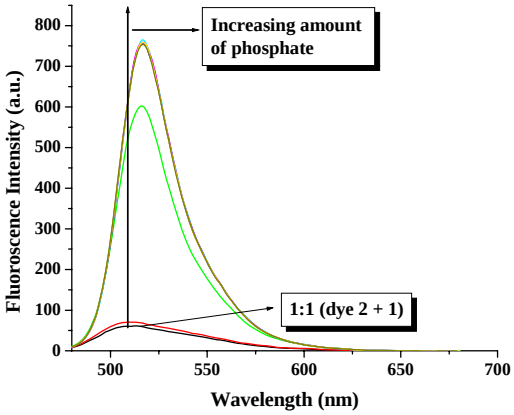


Figure S21: Change in fluorescence intensity due to gradual addition of phosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

.

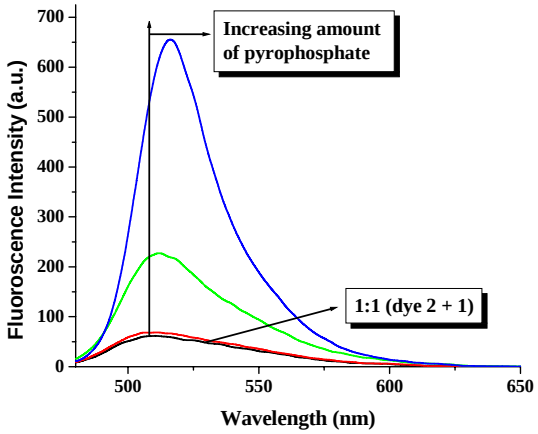


Figure S22: Change in fluorescence intensity due to gradual addition of AMP (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

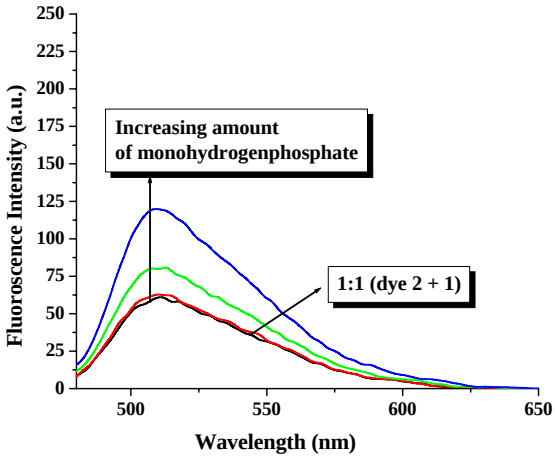


Figure S23: Change in fluorescence intensity due to gradual addition of monohydrogenphosphate (c = 1.56 x 10^{-3} M) to the ensemble of dye **2**/**1** (1:1). All titration are performed at 25 ^{0}C in H_2O (pH = 6.4, 10 mM TrisHCl buffer).

c) Color change in the "Indicator displacement assay"

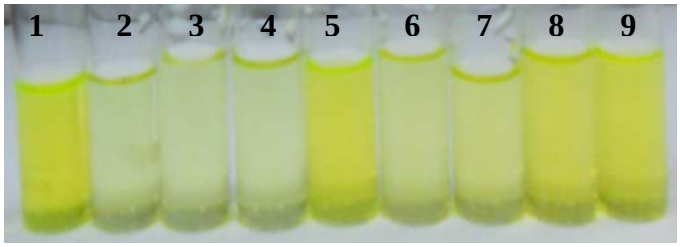


Figure S24: (1) Dye, (2) dye **2** + **1** (1:1) = A, (3) A + ATP, (4) A + ADP, (5) A + AMP, (6) A+ $H_2PO_4^-$, (7) A+ HPO_4^{2-} , (8) A + PO_4^{3-} and (9) pyrophosphate.{ [**1**] = 1.6 x 10^{-3} M, [**2**] = 4.2 x 10^{-5} M, [G] = 1.56 X 10^{-3}, in H_2O (pH = 6.4, 10 mM Tris/HCl buffer) at 25 ^{0}C}

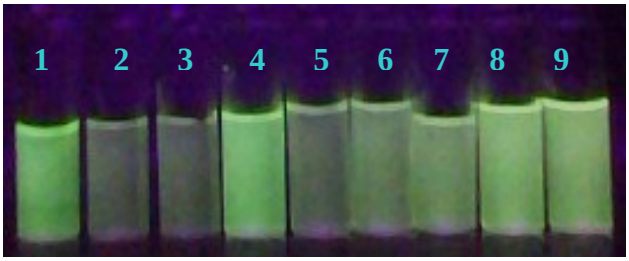


Figure S25: (1) Dye, (2) dye **2** + **1** (1:1) = A, (3) A + ATP, (4) A + AMP, (5) A + ADP, (6) A+ $H_2PO_4^-$, (7) A+ HPO_4^{2-} , (8) A + PO_4^{3-} and (9) pyrophosphate, while respective solution in vials in figure irradiated at 365 nm

d) **Job plot of 2 with 1 from fluorescence**

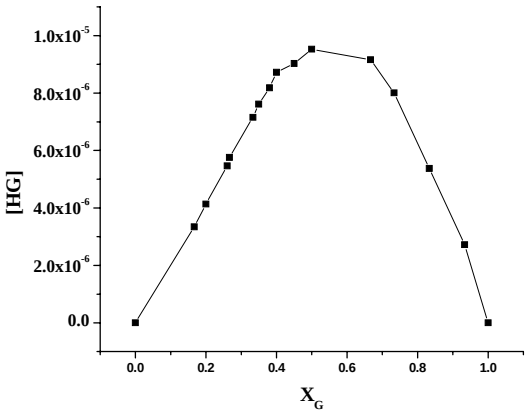


Figure S26: Job plot of dye **2** with **1** from fluorescence at 511 nm in H_2O (pH = 6.4, 10 mM TrisHCl buffer). [G] = [H] = 5.0 x 10^{-5} M.

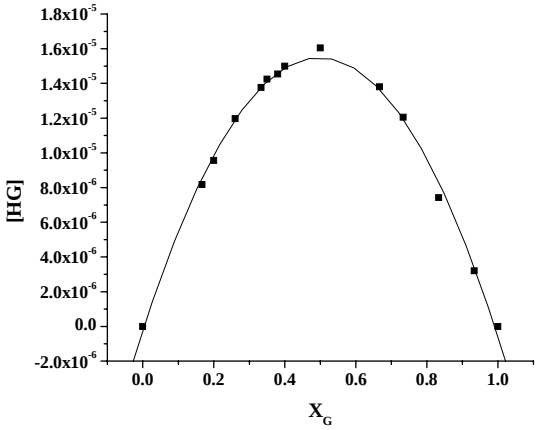


Figure S27: Job plot of dye **2** with **1** from UV at 485 nm in H_2O (pH = 6.4, 10 mM TrisHCl buffer). [G] = [H] = 5.0 x 10^{-5} M.

e) Binding constant curves for 1 with the anions in H_2O (pH = 6.4, 10mM TrisHCl buffer)

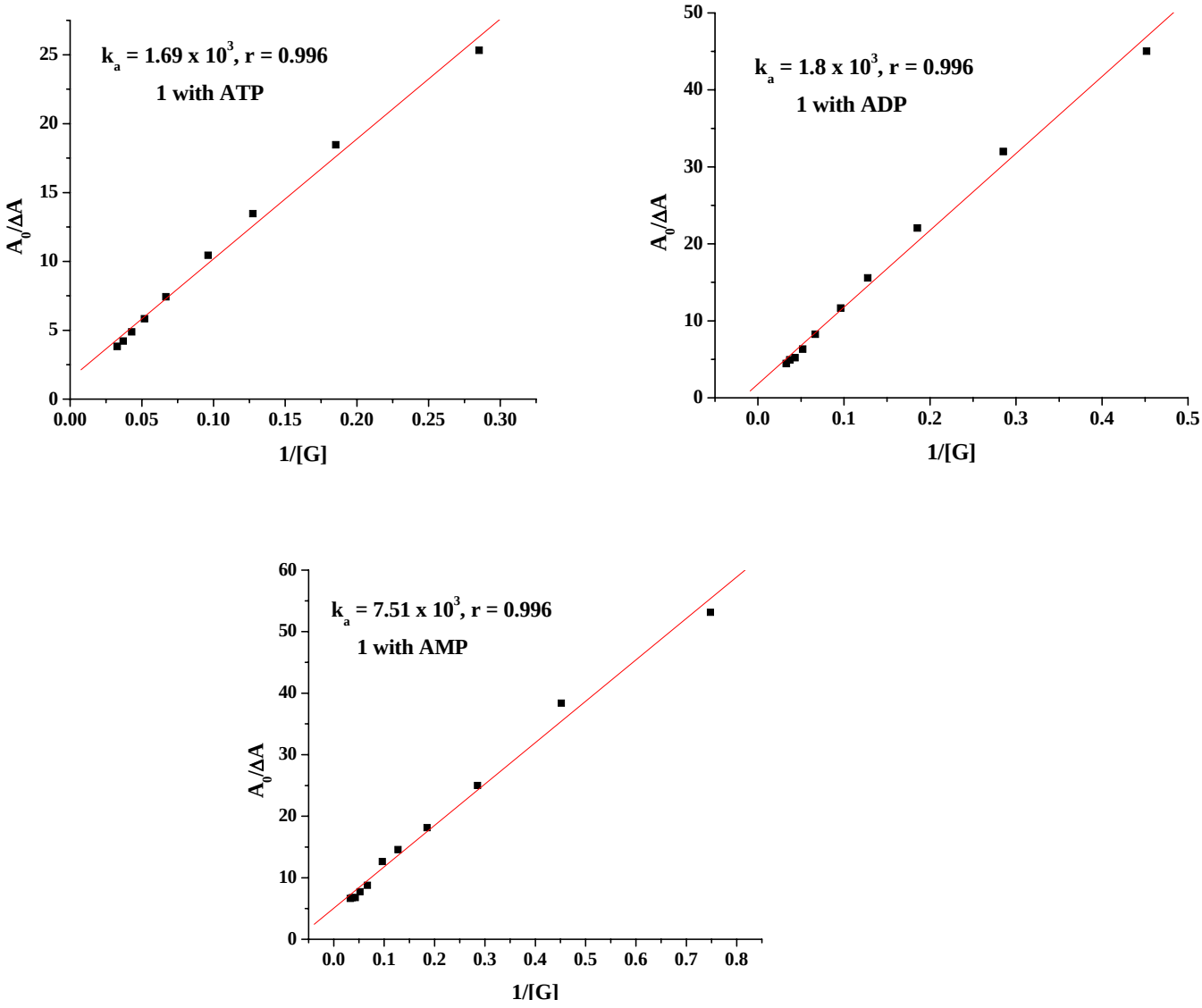


Figure S28: Binding constant curves of **1** (c = 6.7 x 10^{-5} M) from UV-vis titration with Na salt of (a) ATP, (b) ADP and (c) AMP in H_2O (pH = 6.4, 10 mM TrisHCl Buffer). [G] = 1.35 x 10^{-3} M.

f) Binding constant curves of 1 with the dye 2 in H_2O (pH = 6.4, 10mM TrisHCl buffer)

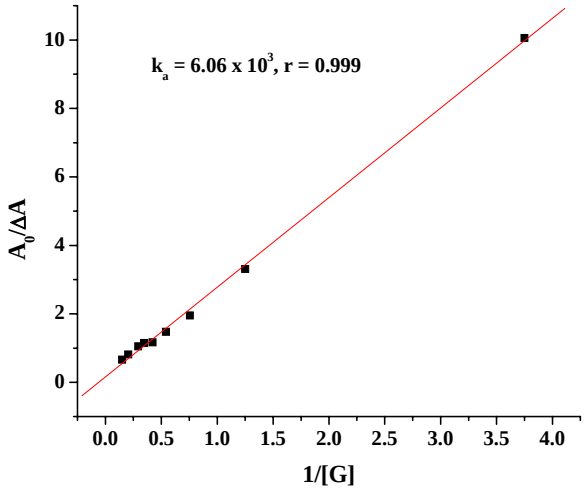


Figure S 29: Binding constant curves of dye **2** (c = 4.2 x 10^{-5} M) with **1** (c = 1.6 x 10^{-3} M) from UV-vis titration in H_2O (pH = 6.43, 10 mM TrisHCl Buffer).

g)

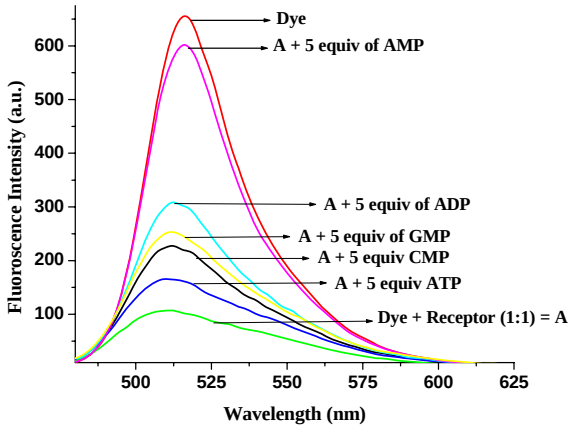


Figure S30: Change in fluorescence of dye in H_2O upon addition of equiv. amount of **1** and other guests in 5 equiv. amounts to the ensemble of **1**/dye **2** (pH = 6.4, 10 mM TrisHCl buffer, [dye **2**] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M).

.

h) Selectivity study

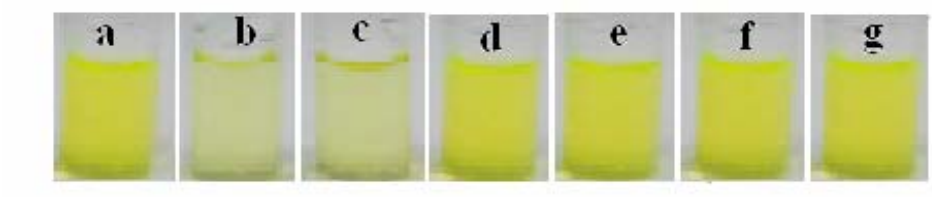


Figure S31: **(**a) dye , (b) receptor **1** + dye (1:1) = **A**, **A** with 5 equiv. amount of (c) ATP + ADP, (d) ADP + ATP + AMP; (e) Phosphate; (f) Pyrophosphate ; (g) Phosphate +Pyrophosphate + AMP in H_2O (pH = 6.4, 10 mM TrisHcl Buffer, [dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 4.7 x 10^{-3} M).

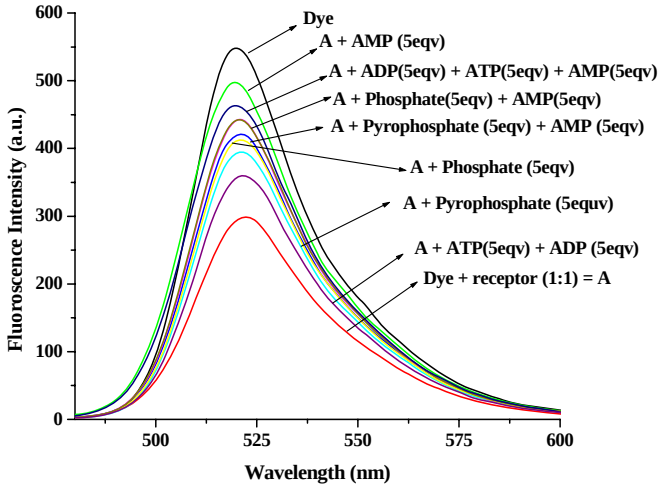


Figure S32: Change in fluorescence intensity of dye upon addition of receptor **1** (1:1) and other guests (5 equiv.) to the ensemble of **1**/dye **2** in H_2O (pH = 6.4, 10 mM TrisHCl Buffer) ([dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 4.7 x 10^{-3} M).

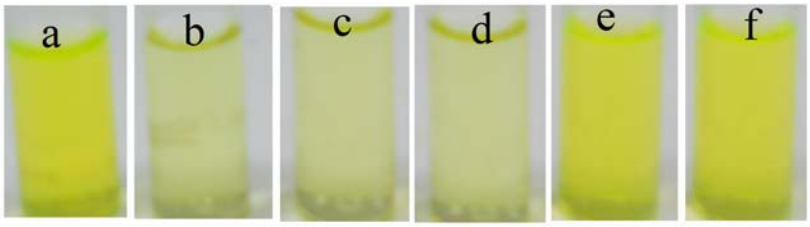


Figure S33: (a) dye **2**, (b) receptor **1** + dye **2** (1:1) = **A**, **A** with 5 equiv. amounts of (c) ADP (d) ATP (e) A + ADP +ALP, (f) A + ATP +ALP ([dye **2**] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M).

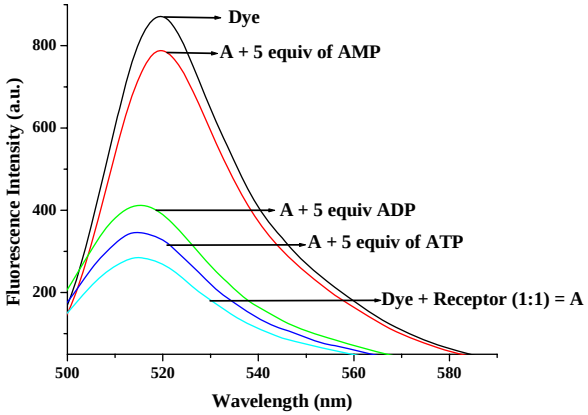


Figure S34: Change in fluorescence intensity of dye upon addition of **1** (1:1) and other guests (5 equiv.) to the ensemble of **1**/dye **2** in H_2O (pH = 7.0, 10 mM TrisHCl Buffer). [dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M.

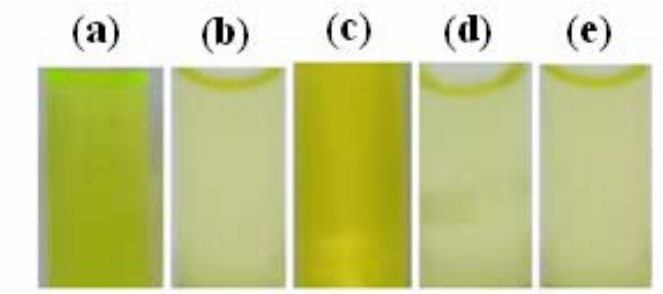


Figure S35: (a) dye, (b) receptor **1** + dye (1:1) = **A**, **A** with 5 equiv. amount of (c) AMP (d) ADP (e) ATP ([dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M) at pH = 7.0.

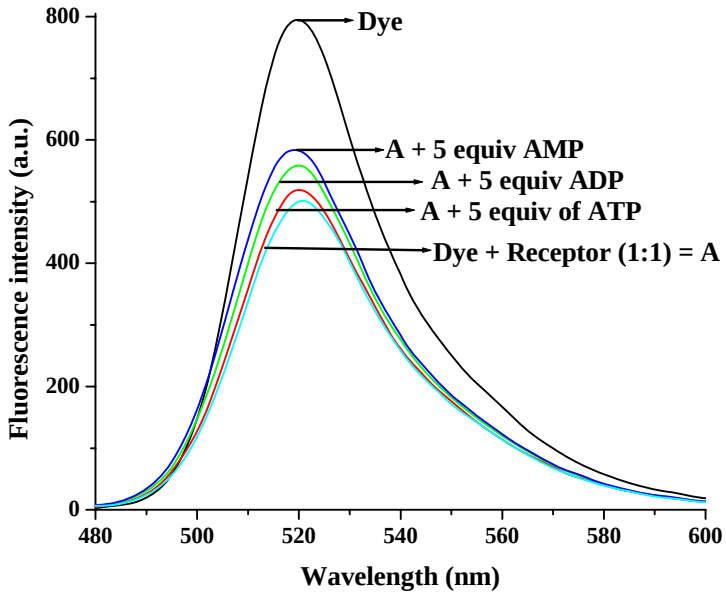


Figure S36: Change in fluorescence intensity of dye upon addition of **1** (1:1) and other guests (5 equiv.) to the ensemble of **1**/dye **2** in H_2O (pH = 8.0, 10 mM TrisHCl Buffer). [dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M.

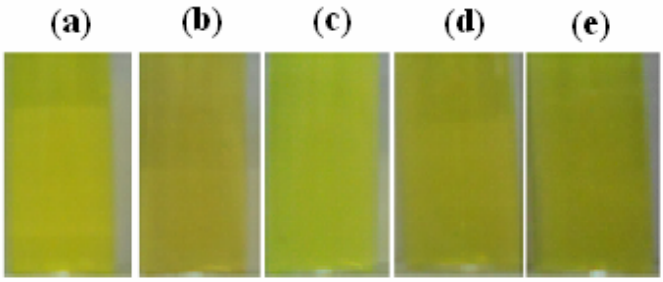


Figure S37: (a) dye, (b) receptor **1** + dye (1:1) = **A**, **A** with 5 equiv. amount of (c) AMP (d) ADP (e) ATP ([dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, [G] = 1.56 x 10^{-3} M) at pH = 8.0.

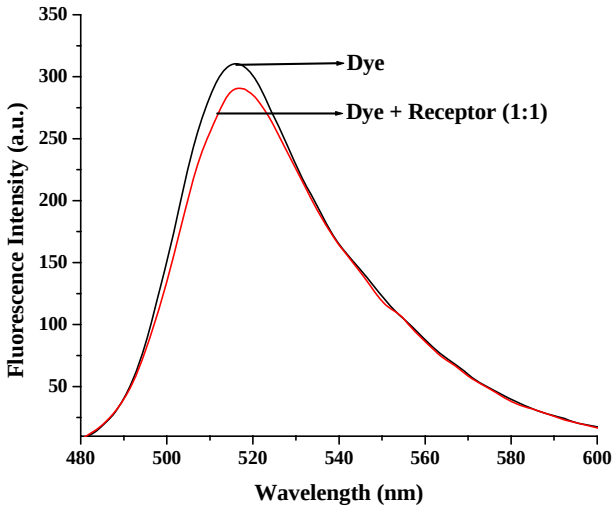


Figure S38: Change in fluorescence intensity of dye upon addition of receptor **1** (1:1) [dye] = 4.2 x 10^{-5} M, [**1**] = 1.6 x 10^{-3} M, at pH = 5.0.

8. MTT assay

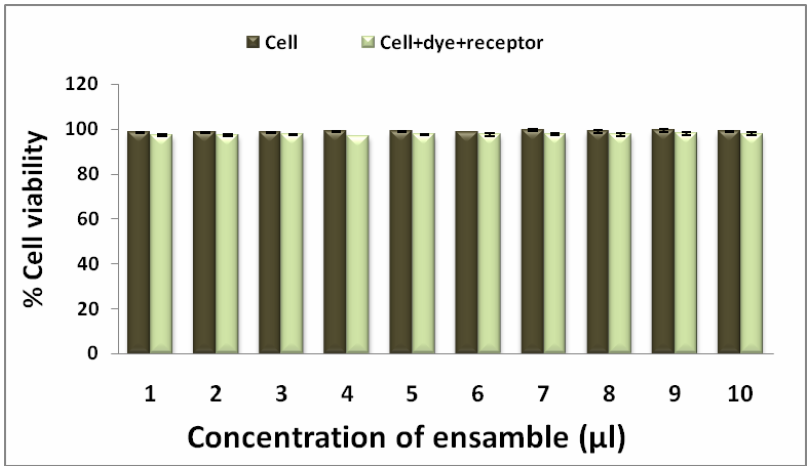


Figure S39. MTT assay.

9. Quantitative analysis of fluorescence by using Fluorescence activated cell sorter (FACS)

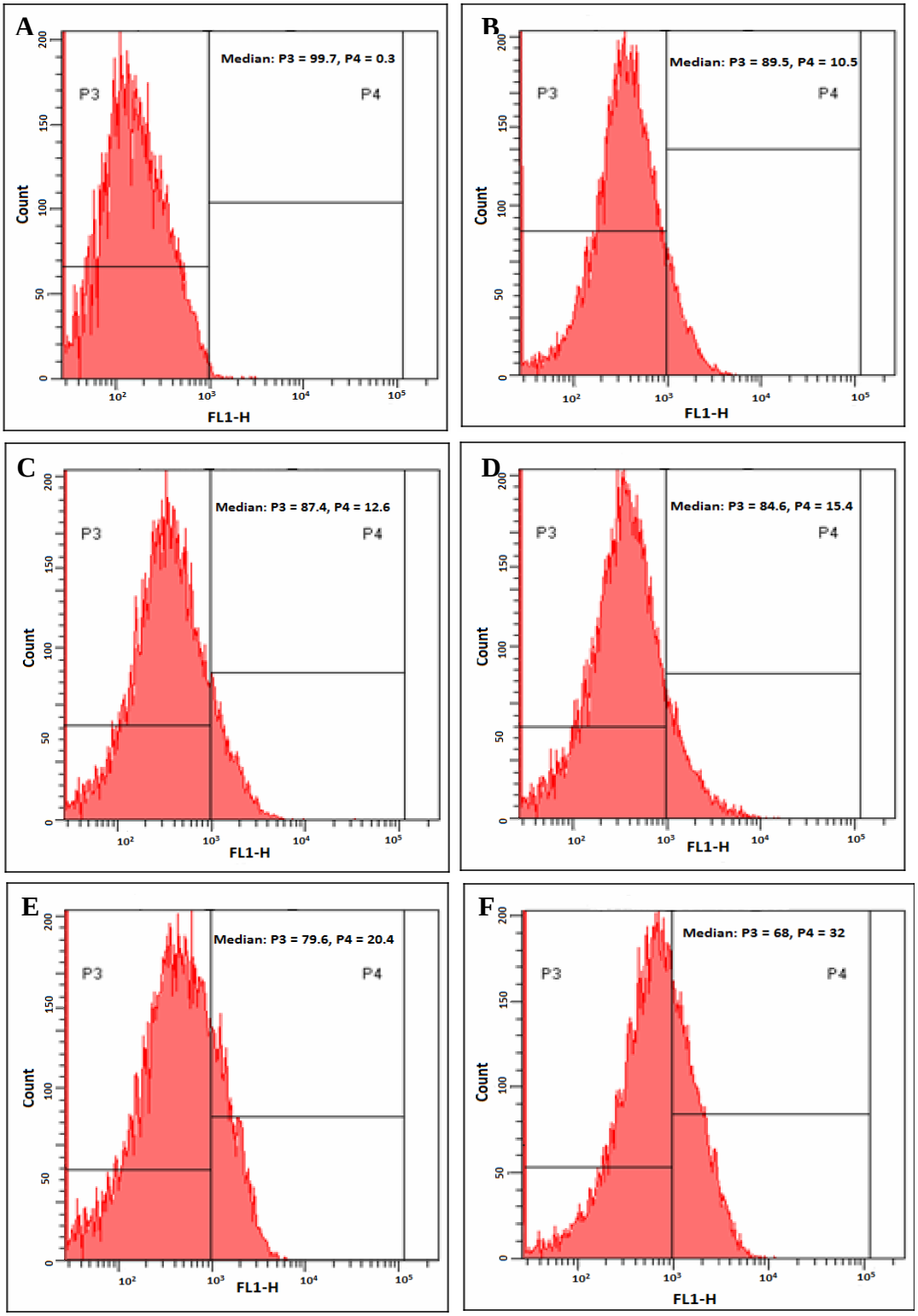


Figure S40: A= cell without receptor **1**, B= cell + ensemble **1**/dye **2**, C= cell + ATP + ensemble **1**/dye **2,** D= cell + ADP + ensemble **1**/dye **2,** E= cell + AMP + ensemble **1**/dye **2** (5 min), F= cell + AMP + ensemble **1**/dye **2** (10 min).

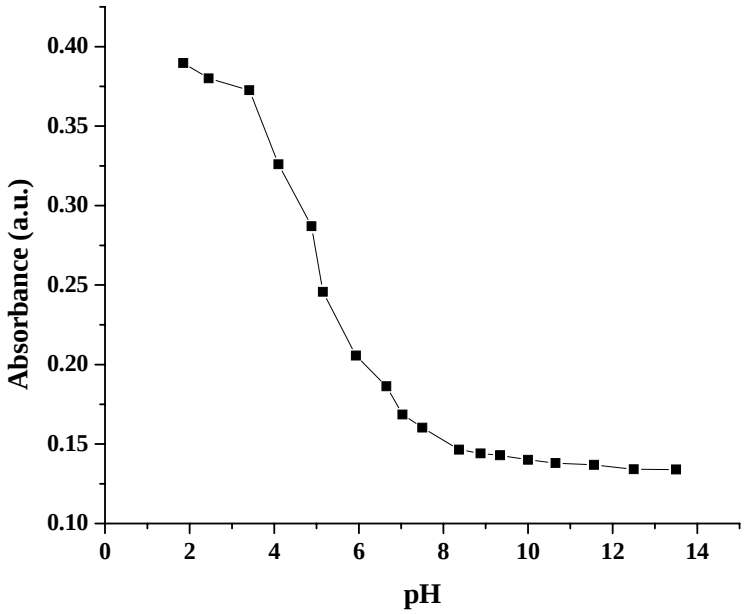
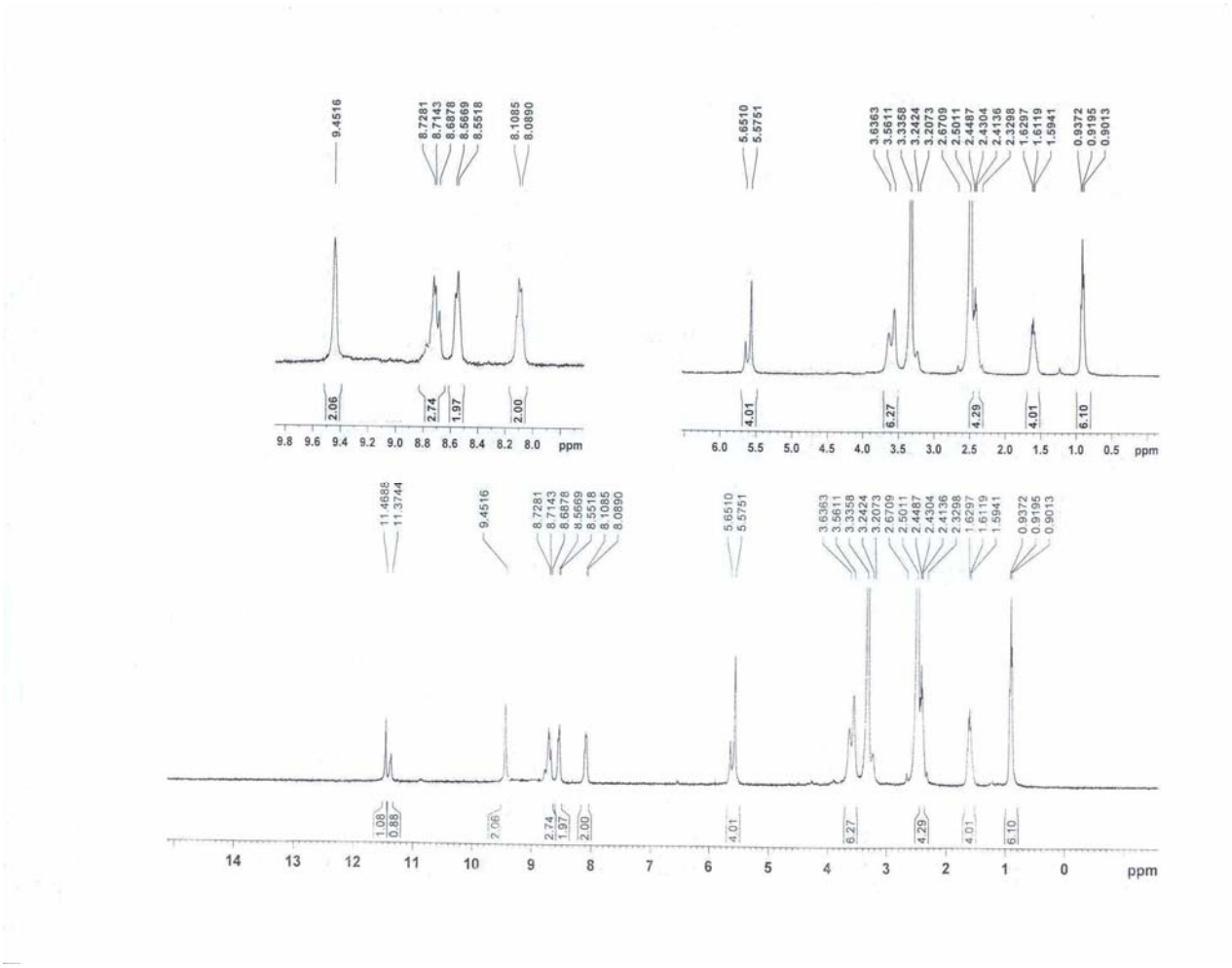


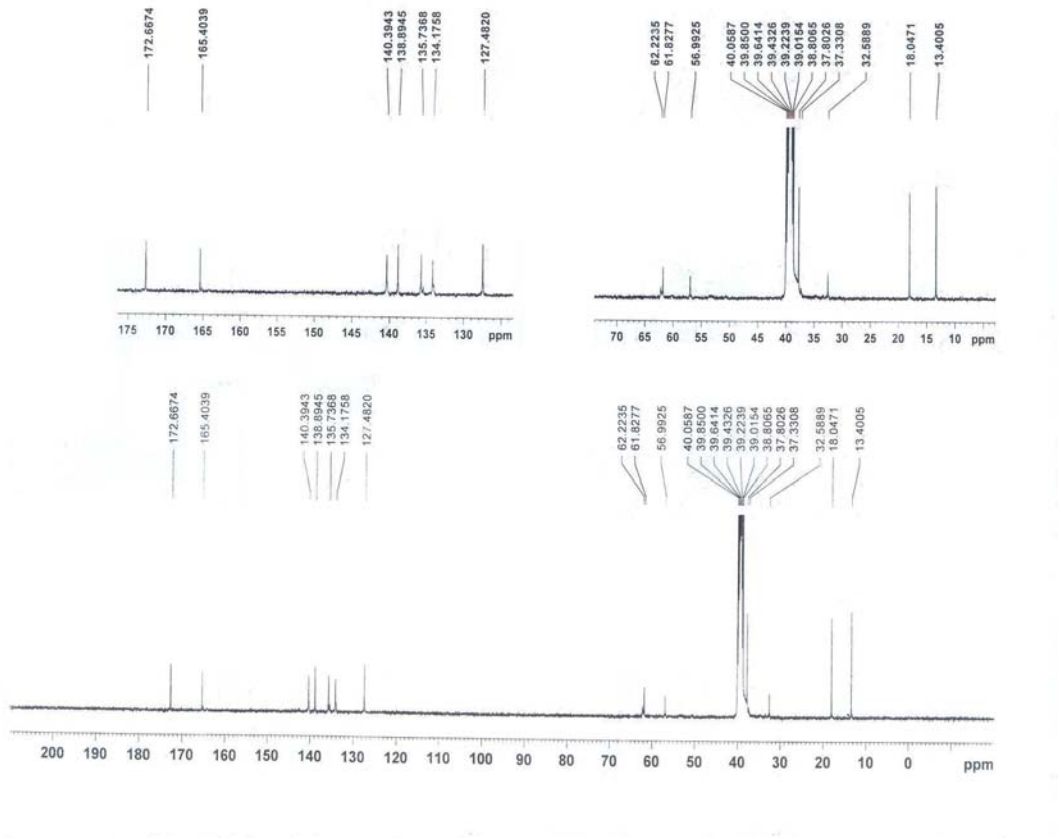
Figure S41: Change in absorbance of **1** with pH in H_2O.

10. Spectral data

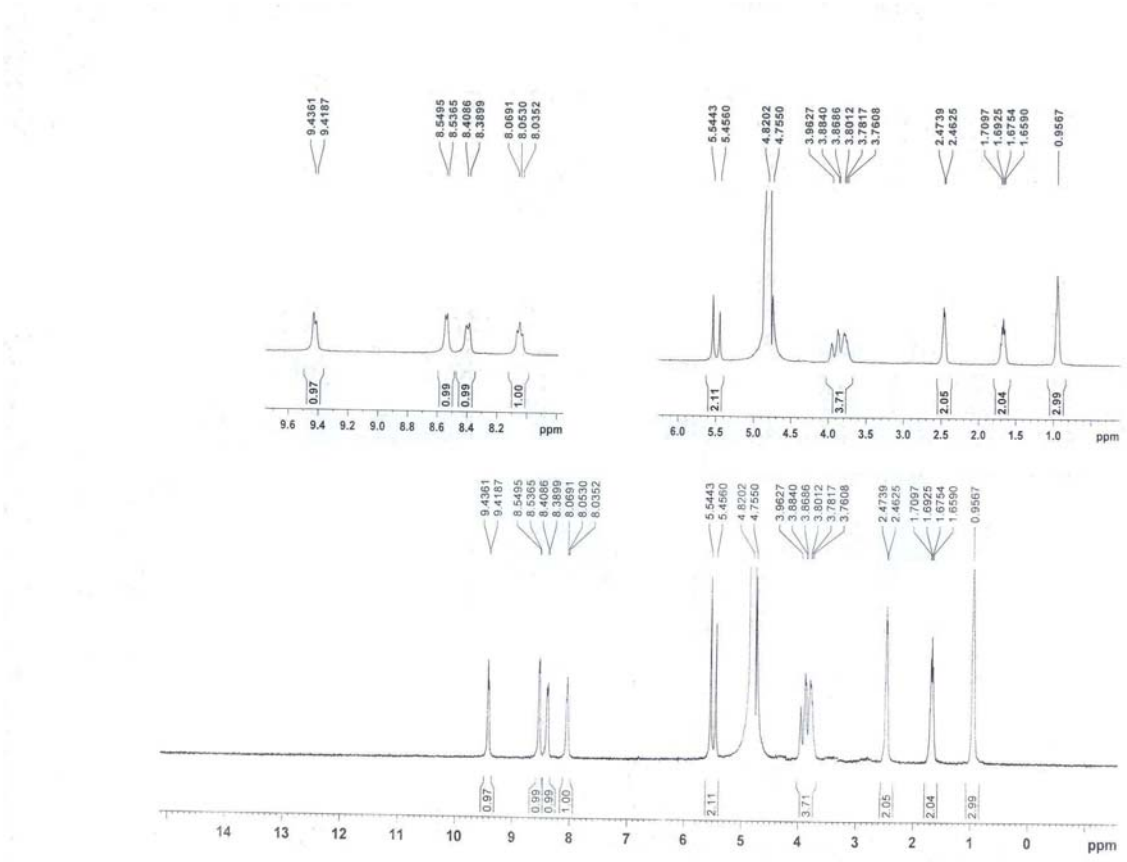
^{1}H NMR (400 MHz, d_6-DMSO)



^{13}C NMR (100 MHz, d_6-DMSO)



^{1}H NMR (400 MHz, D_2O)



HRMS Spectra of receptor **1**