Electronic Supplementary Material (ESI) for New Journal of Chemistry.


# Supporting information

**Dual-mode recognition of biogenic amine tryptamine and fluoride ion by naphthyl hydrazone platform: Application in fluorescence imaging of HeLa cells and Zebrafish embryos**

**Karuppiah Krishnaveni, Sepperumal Murugesan* and Ayyanar Siva***

Supramolecular and Organometallic Chemistry Lab, Department of Inorganic Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai-21

Corresponding authors E-mail address: drasiva@gmail.com; siva.chem@mkuniversity.org (A.Siva); smsan@mail.com; murugesan.chem@mkuniversity.org (S. Murugesan)

**1. Determination of Binding constant from Benesi-hildebrand method**

The binding constant of $F^-$ with PYNA has been calculated by using UV-Visible and Fluorescence spectrometer respectively. The fixed concentration of PYNA was used throughout the titration and any given concentration of $F^-$ with PYNA gives good linear relationship. The binding constant value of $F^-$ with PYNA was determined by using Benesi-Hildebrand eqn[1].

**$1/ (A-Ao) = 1/ \{K (Amax–Ao) [F^-]\} + 1/ [Amax-Ao]$**

Here, Ao is the absorbance of PYNA without $F^-$ ions, A is the absorbance of PYNA with $F^-$ ions (at given concentration), Amax is the absorbance of PYNA with $F^-$ ions (in saturated concentration). K is the association constant ($M^{-1}$). The association constant (K) could be determined from the slope of plot 1/ (A-Ao) VS 1/ $[F^-]$.

Further, the binding constant values of Tryptamine (TryptA) and $F^-$ with PYNA have been calculated by using a fluorescence method. The concentration of PYNA was kept constant throughout the titration and varying the concentration of the TryptA and $F^-$ gives good linear relationship. The binding constant value of TryptA/ $F^-$ with PYNA was calculated from by using modified Benesi - Hildebrand equation[2].

**$1/I-Imin = 1/ Imax-Imin + (1/K[C]) (1/ Imax-Imin)$**

Here, Imin is the emission intensity of PYNA without TryptA, I is the emission intensity of PYNA with any given concentration of TryptA, Imax is the emission intensity of PYNA at a concentration of complete saturation, K is the binding constant,[C] is the concentration of PYNA. The value of K has been determined from the slope of the plot (Imax-Imin) / (I-Imin) VS 1/[C] for PYNA-TryptA.

**2. Determination of Limit of Detection (LOD)**

The limit of detection was calculated using this equation[3].

**DL = CL× CT**

where **CL** is the Conc. of Ligand, **CT** is the Conc. of Titrant at which changes are observed.

Thus for TryptA; $DL = 4\times10^{-5} \times 0.1\times10^{-6} = 0.4 \times10^{-11} = 0.004 \times10^{-8}$ and for $F^-$ ions; $DL = 6\times10^{-5} \times 0.05\times10^{-6} = 0.3\times10^{-11} = 0.003\times10^{-8}$

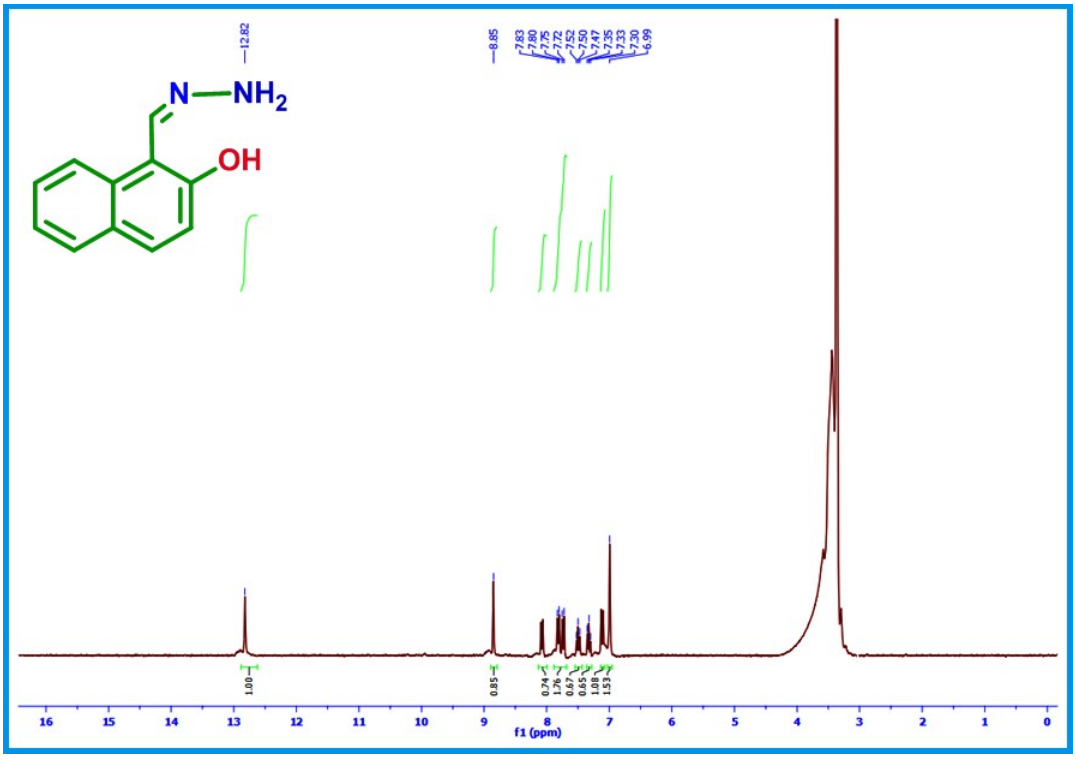



**Fig- S1: $^{1}$H NMR spectrum of NAHZ in DMSO-d$^{6}$**

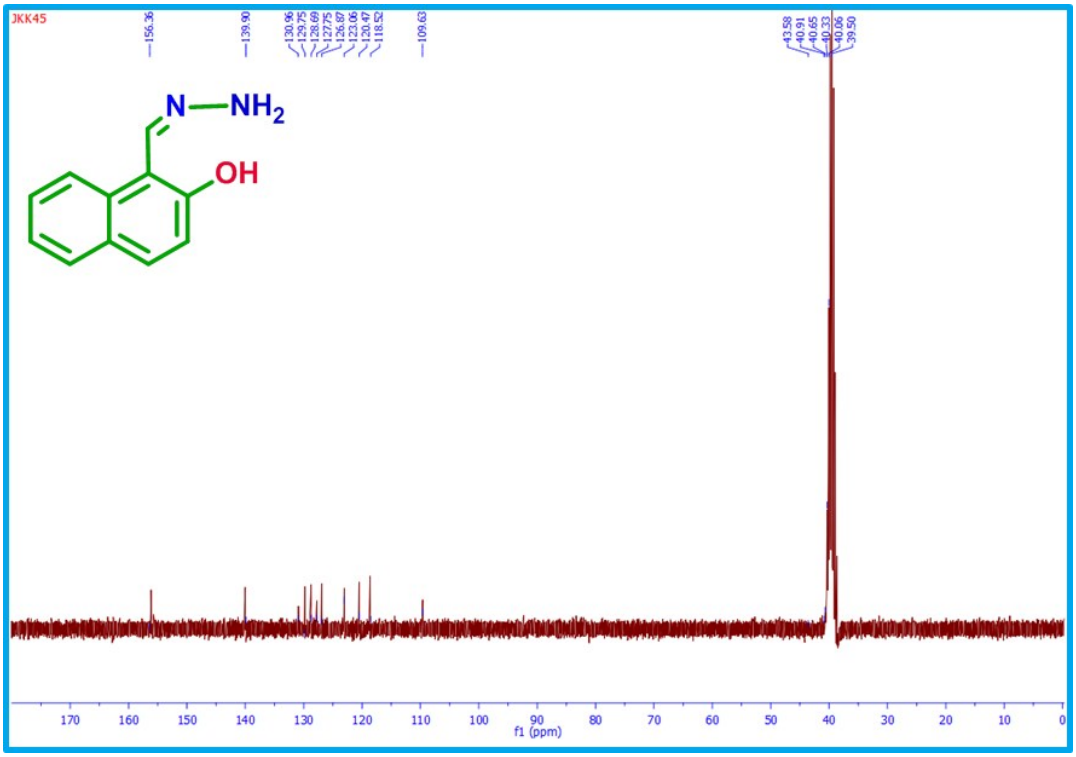



**Fig- S2: $^{13}$C NMR spectrum of NAHZ in DMSO-d$^{6}$**

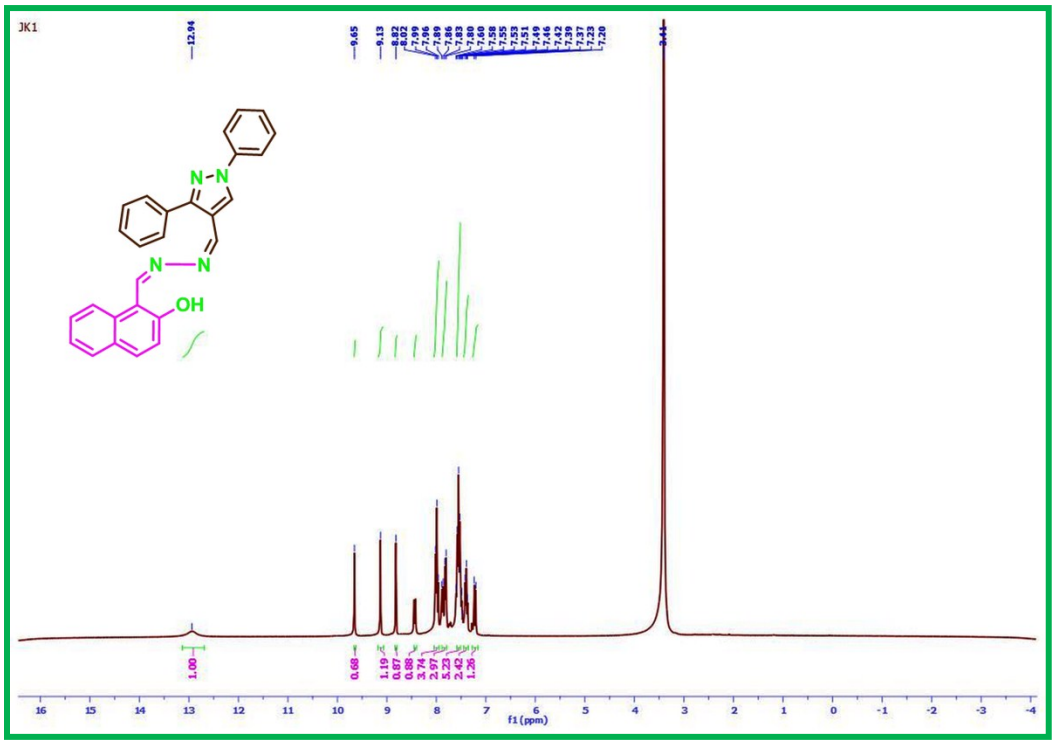



**Fig- S3: $^{1}$H NMR spectrum of PYNA in DMSO- $d_6$**

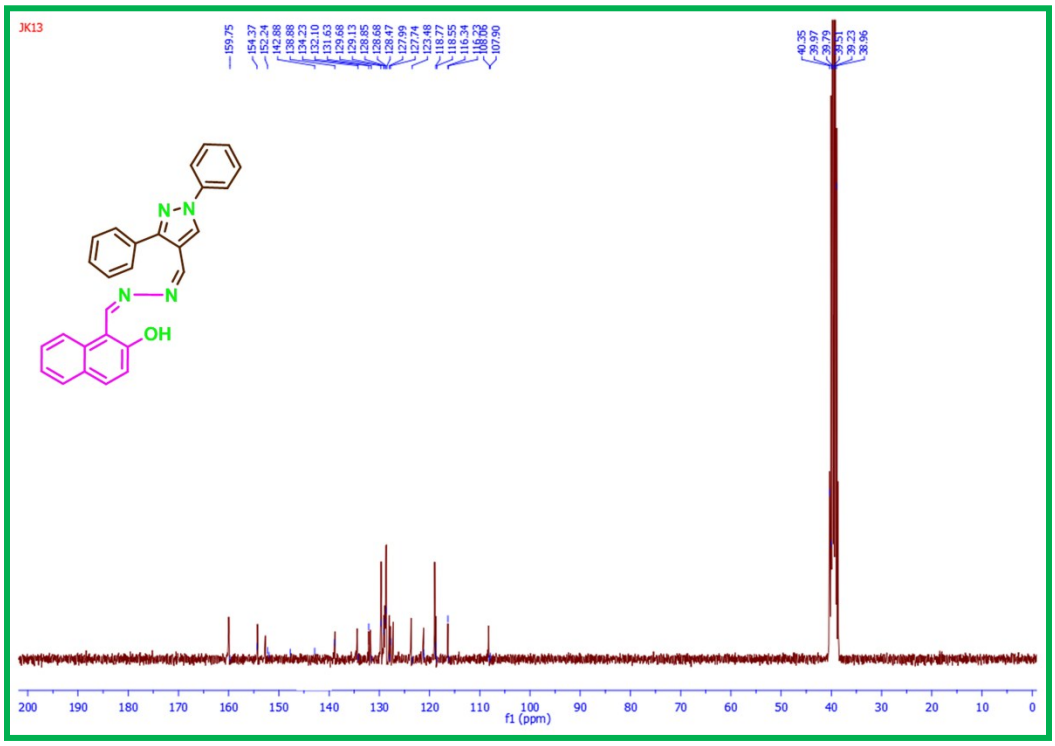



**Fig- S4: $^{13}$C NMR spectrum of PYNA in DMSO- $d_6$**

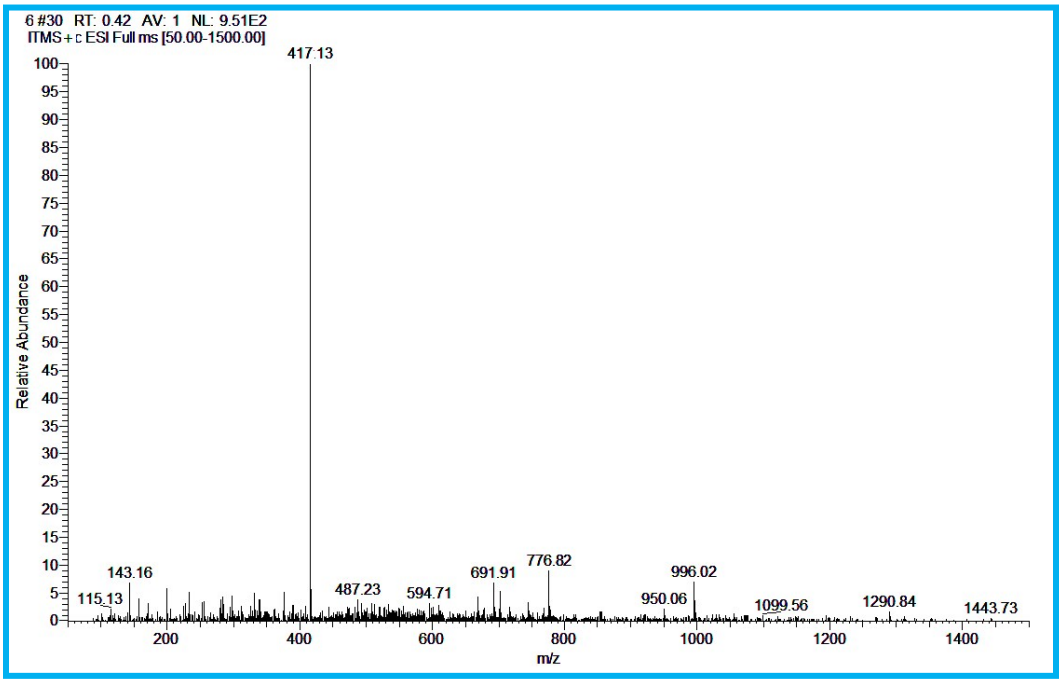



**Fig- S5: ESI-MS spectrum of PYNA**

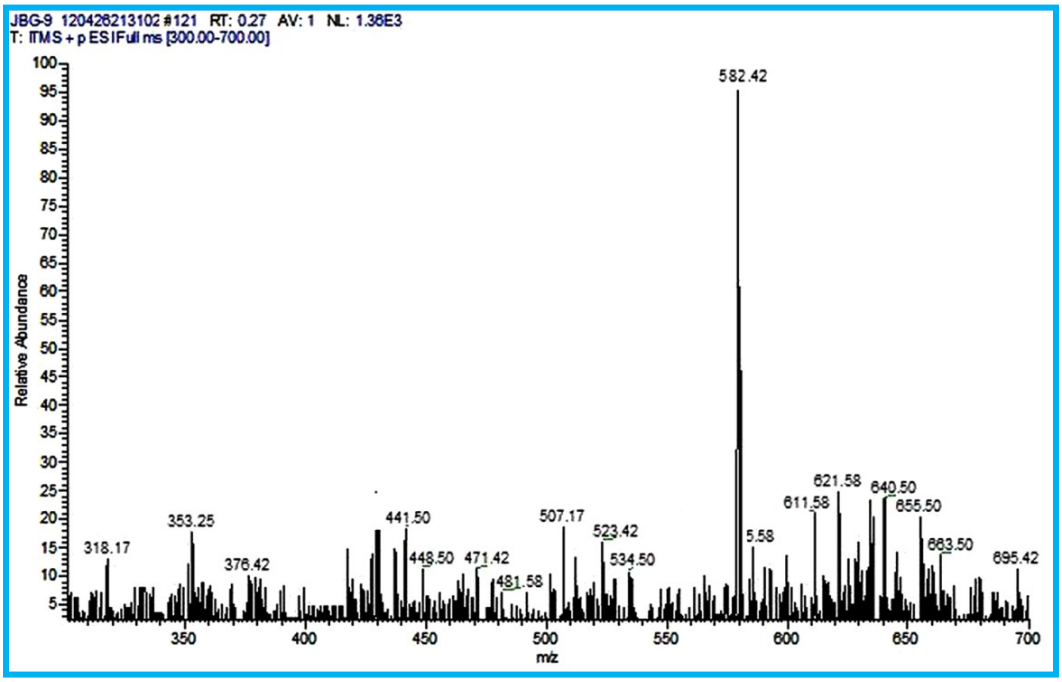



**Fig- S6: ESI-MS spectrum of PYNA-TryptA**

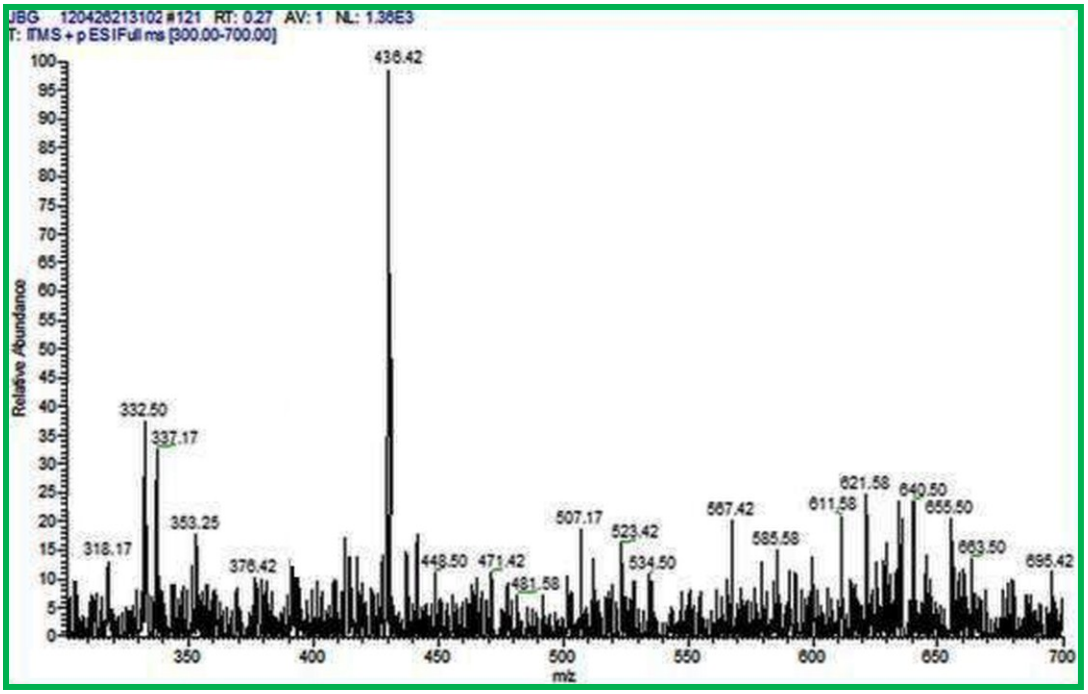
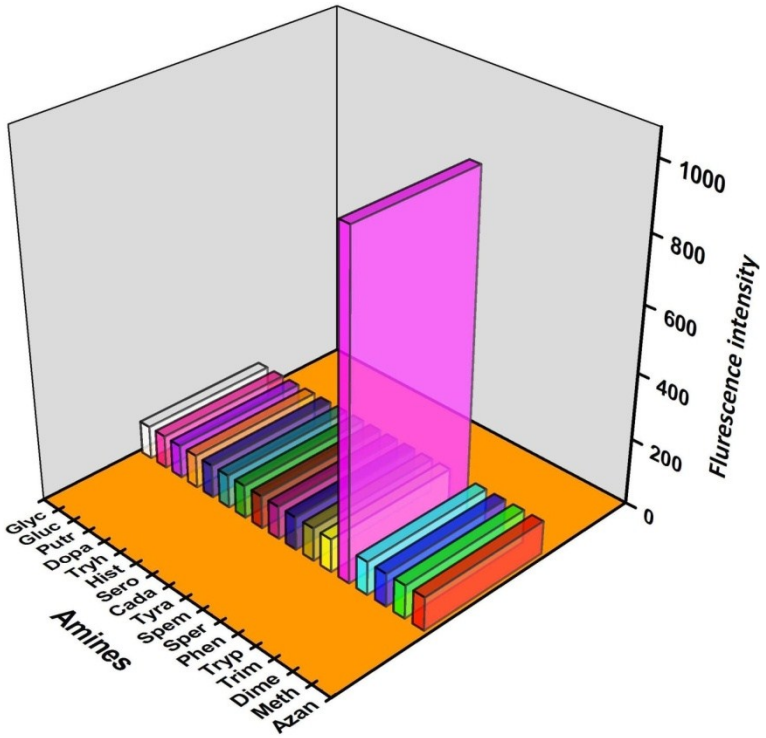



**Fig- S7: ESI-MS spectrum of PYNA- $F^-$**



**(a)**

**(b)**

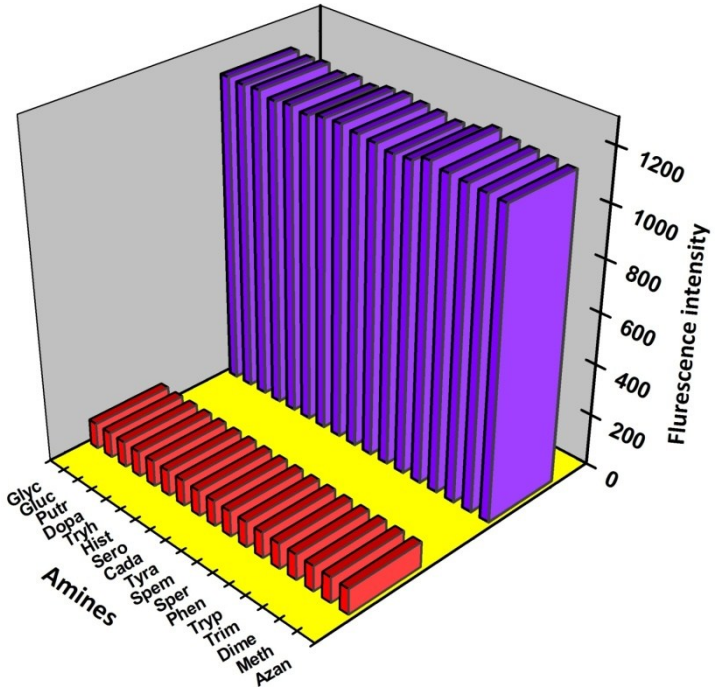
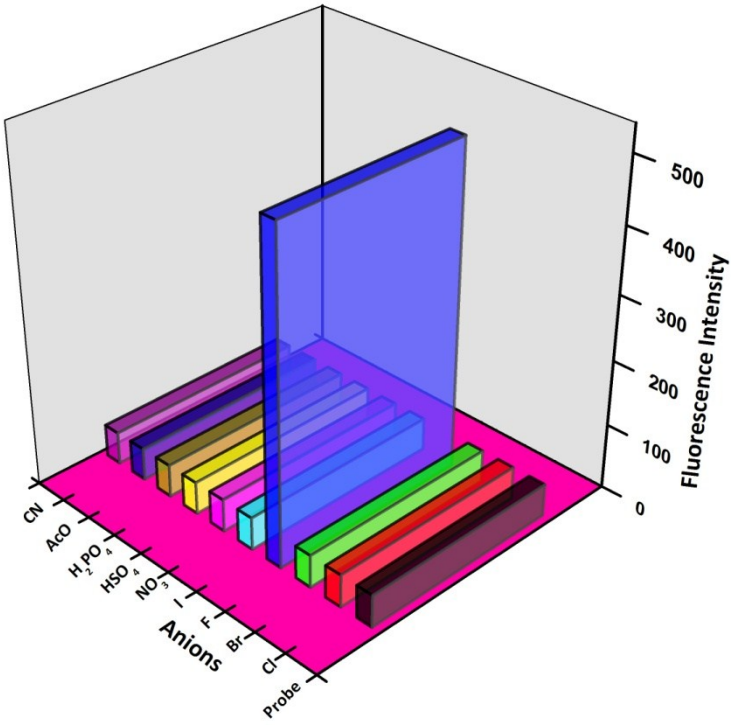



**Fig- S8: (a) Competitive studies of tryptamine with PYNA in presence of other anions (b) The red bars represent the change of the fluorescence intensity of PYNA that occurs upon the consequent addition of competitive amines. The violet bars represent the addition of the competing amines to PYNA. Excitation at 440nm, slit width = 5nm.**

**(a)**




**(b)**

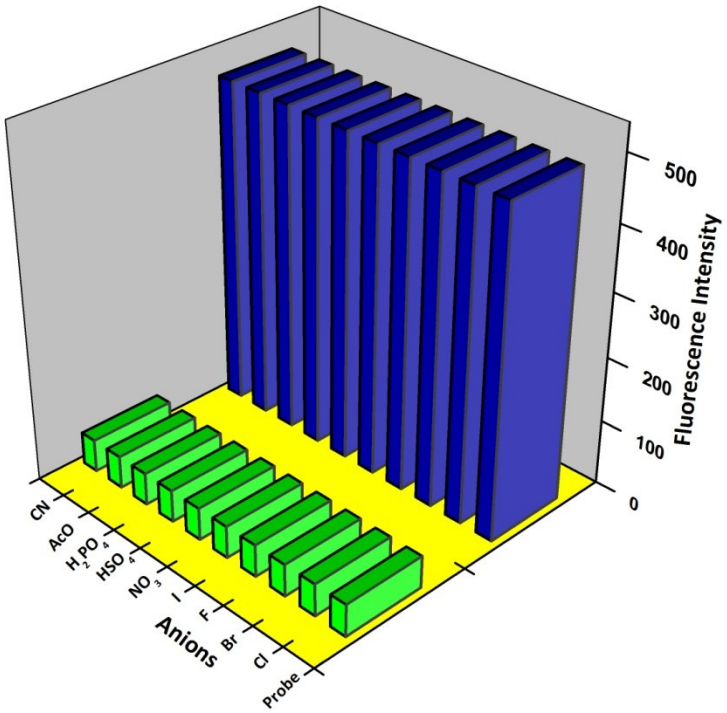



**Fig- S9: (a) Competitive studies of $F^-$ ions with PYNA in presence of other anions (b) The green bars represent the change of the fluorescence intensity of PYNA that occurs upon the consequent addition of other anions. The blue bars represent the addition of the competing anions to PYNA. Excitation at 440nm, slit width = 5nm.**

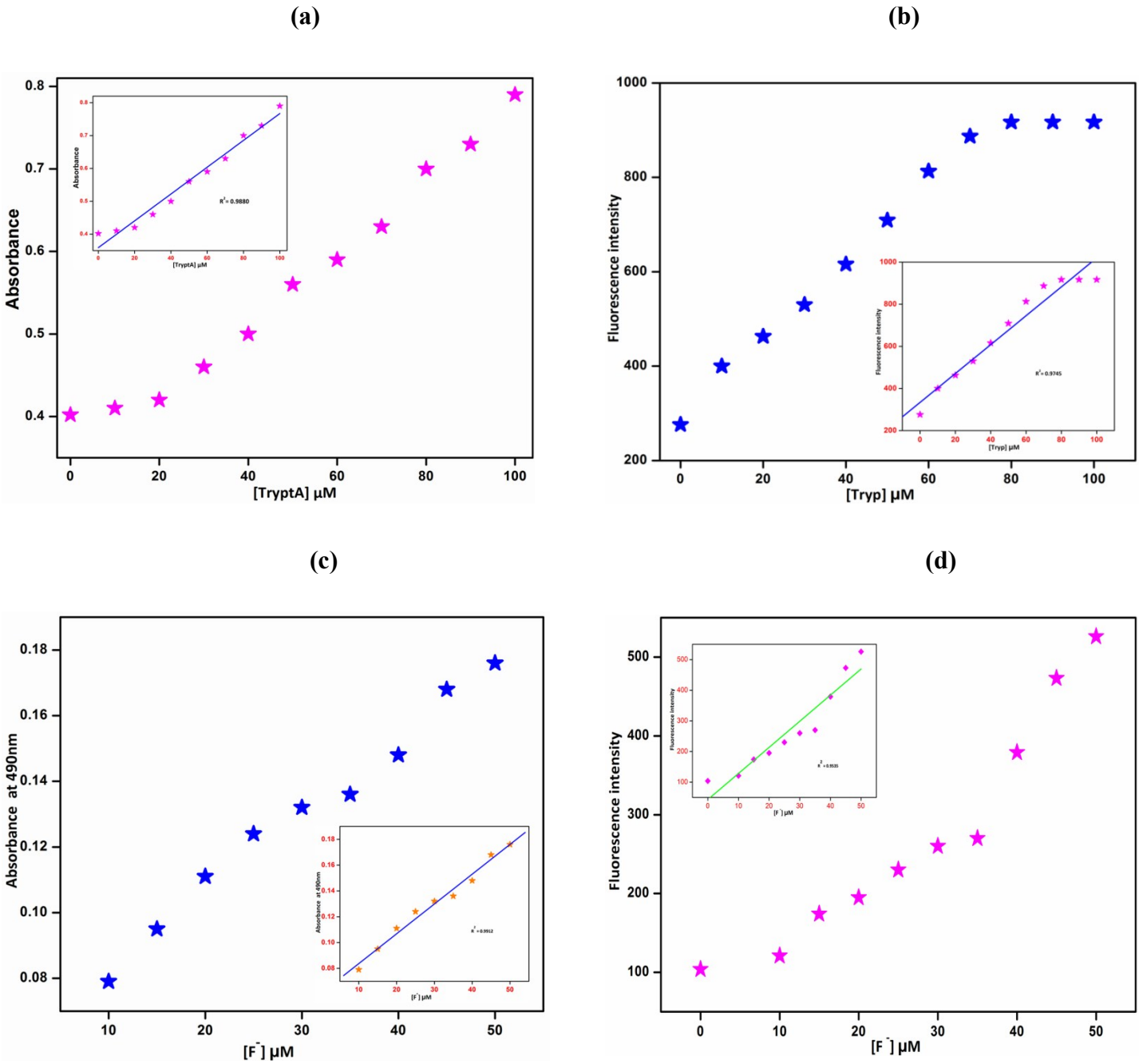



**Fig- S10: (a) Linear fit analysis of PYNA VS TryptA in UV-Visible spectroscopy (Insert figure is fitted linear plot). Absorbance measured at 280 nm. (b)Linear fit analysis of PYNA Vs TryptA in Fluorescence spectroscopy (Insert figure is fitted linear plot). Fluorescence measured at 440 nm. (c) Linear fit analysis of PYNA VS $F^-$ in UV-Visible spectroscopy (Insert figure is fitted linear plot). Absorbance measured at 490 nm. (d) Linear fit analysis of PYNA Vs $F^-$ in Fluorescence spectroscopy (Insert figure is fitted linear plot). Fluorescence measured at 441 nm.**

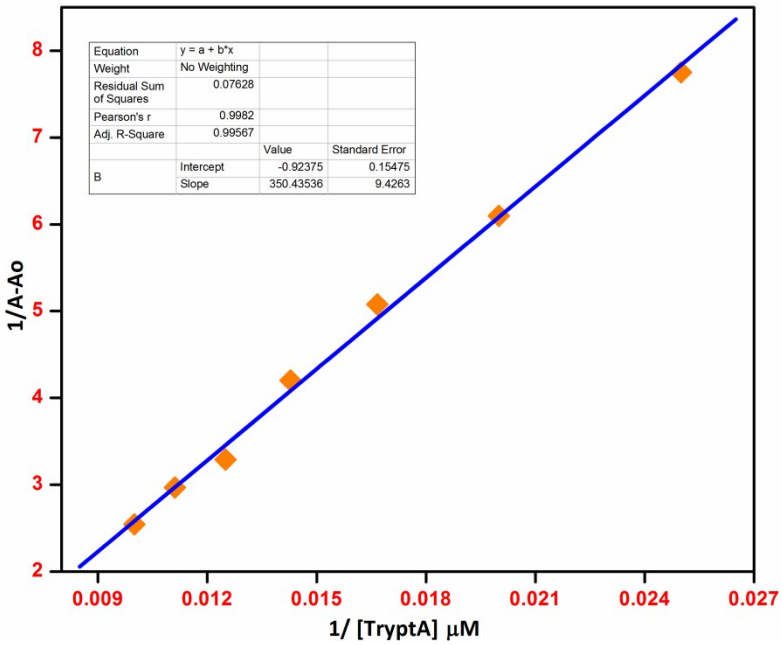



**Fig- S11 (a): Measuring of binding constant value of TyptA with PYNA by B-H plot method from UV-Visible titration profile. Absorbance measured at 280 nm.**

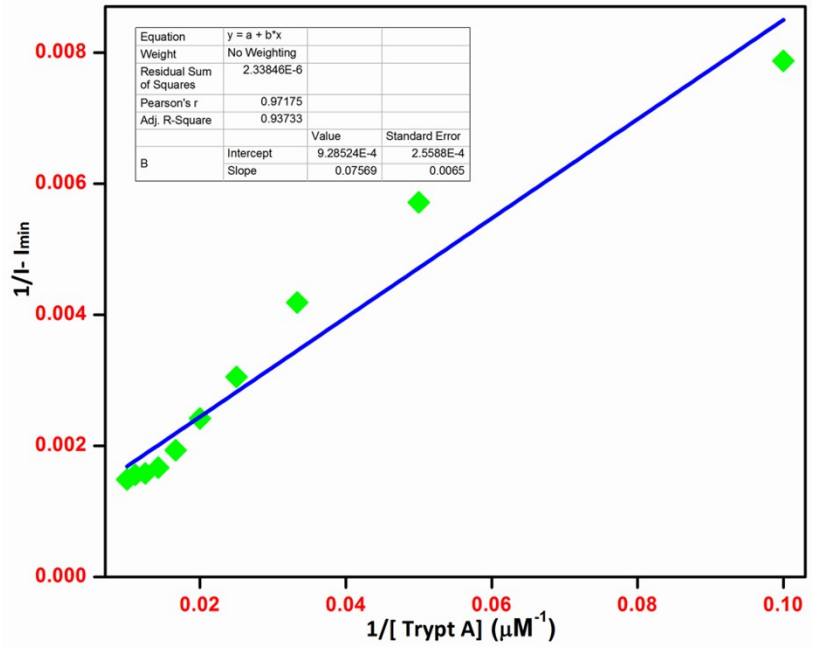



**Fig- S11 (b): Measuring of binding constant value of TyptA with PYNA by B-H plot method from fluorescence titration profile. Fluorescence intensity measured at 441 nm.**

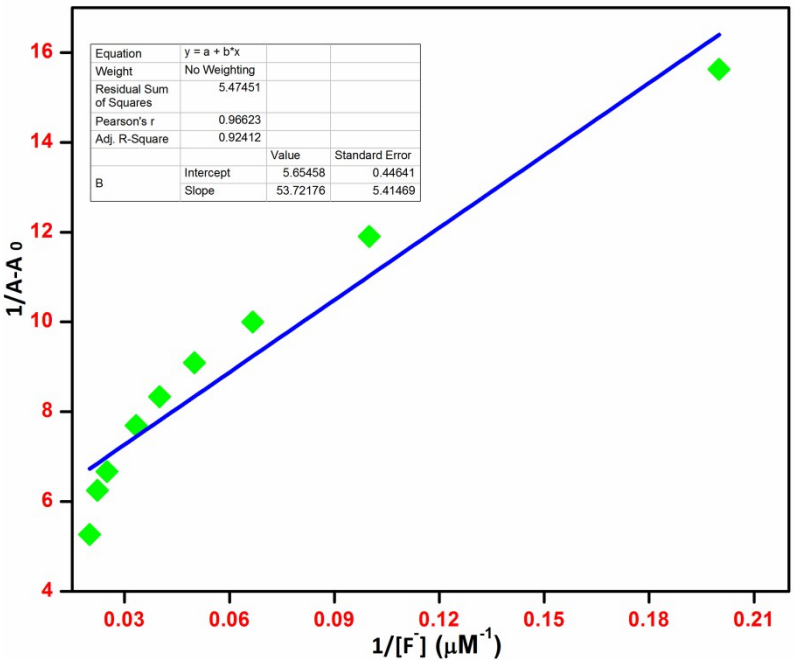



**Fig- S12 (a): Measuring of binding constant value of $F^-$ with PYNA by B-H plot method from UV-Visible titration profile. Absorbance measured at 490 nm.**

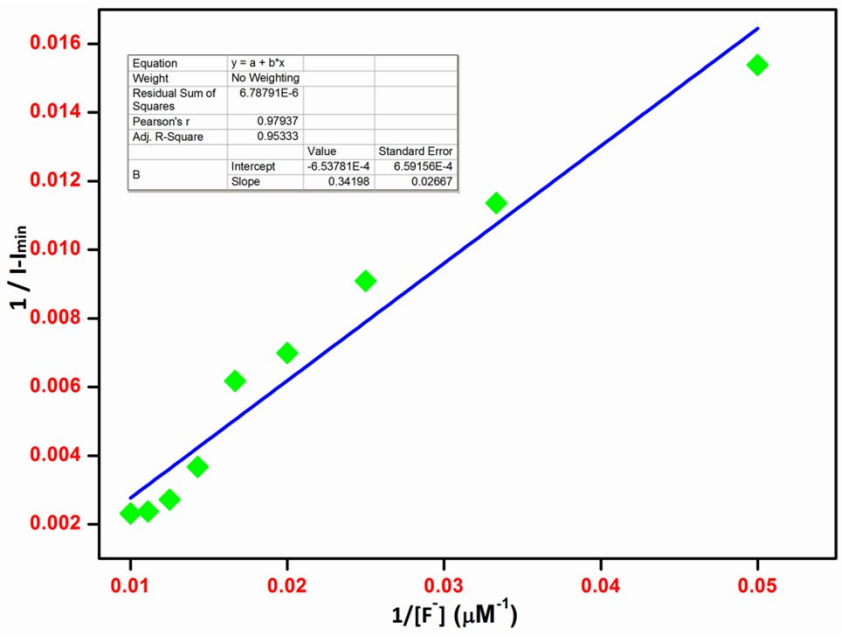



**Fig- S12 (b): Measuring of binding constant value of $F^-$ with PYNA by B-H plot method from fluorescence titration profile. Fluorescence intensity measured at 441 nm.**

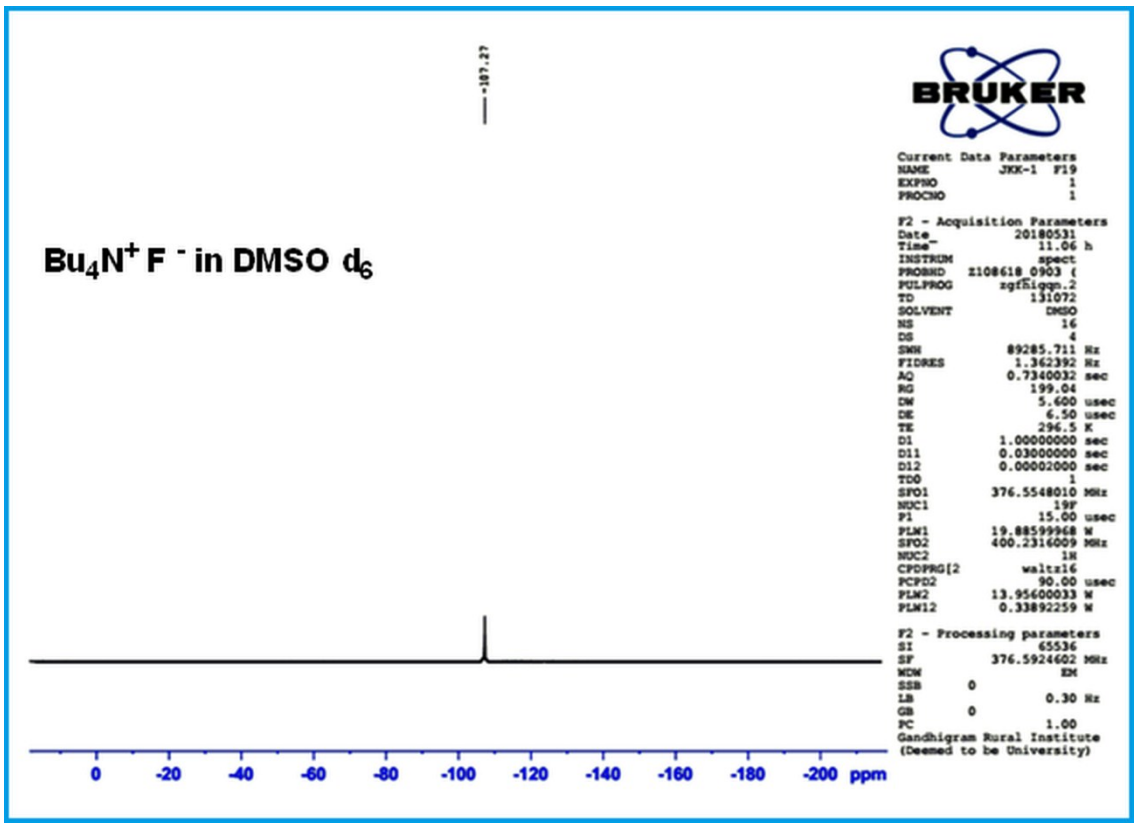



**Fig- S13: $^{19}$F NMR spectrum of Tetrabutylammonium fluoride in DMSO-d$_6$**

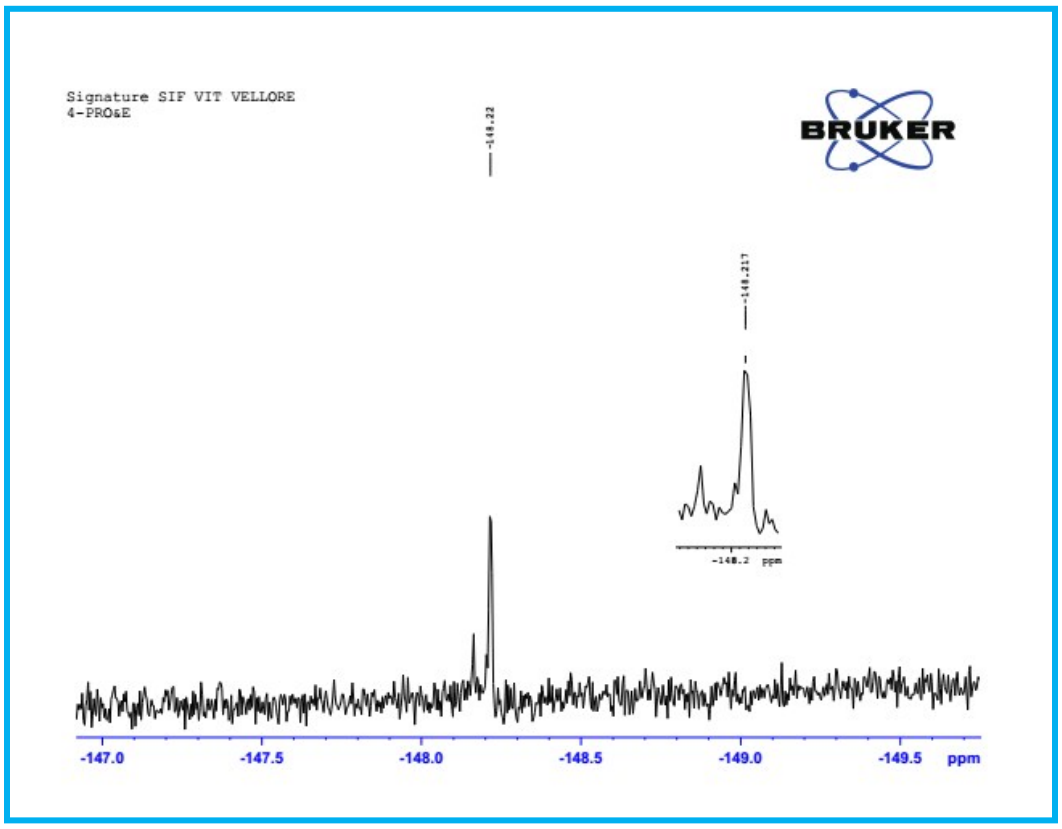



**Fig- S14: $^{19}$F NMR spectrum of fluoride anion interaction with probe PYNA in DMSO-d$_6$**

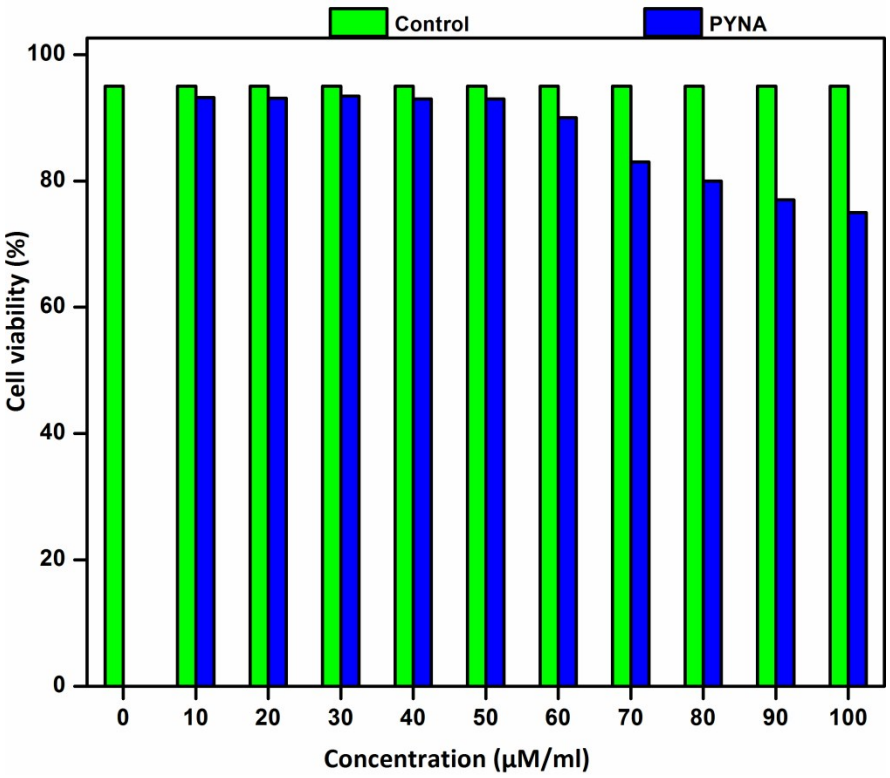



**Fig- S15: Cytotoxicity measurement of PYNA VS HeLa cells**

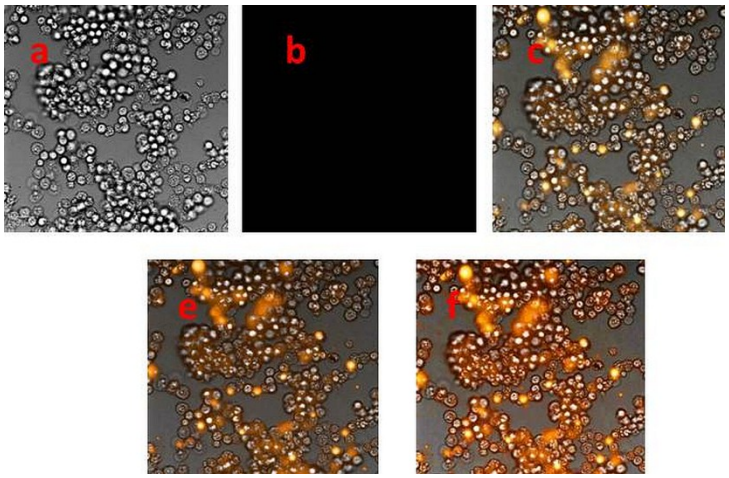



**Fig-S16: Live cell imaging analysis of $F^-$ ion in Human HeLa cell line. (a) Bright field images of PYNA alone (50 µM) (b) fluorescence merged image of PYNA and in the presence of $F^-$ (c) incubated with 10 µM of $F^-$ ions (d) incubated with 25 µM of $F^-$ ions (e) incubated with 50 µM of $F^-$ ions.**

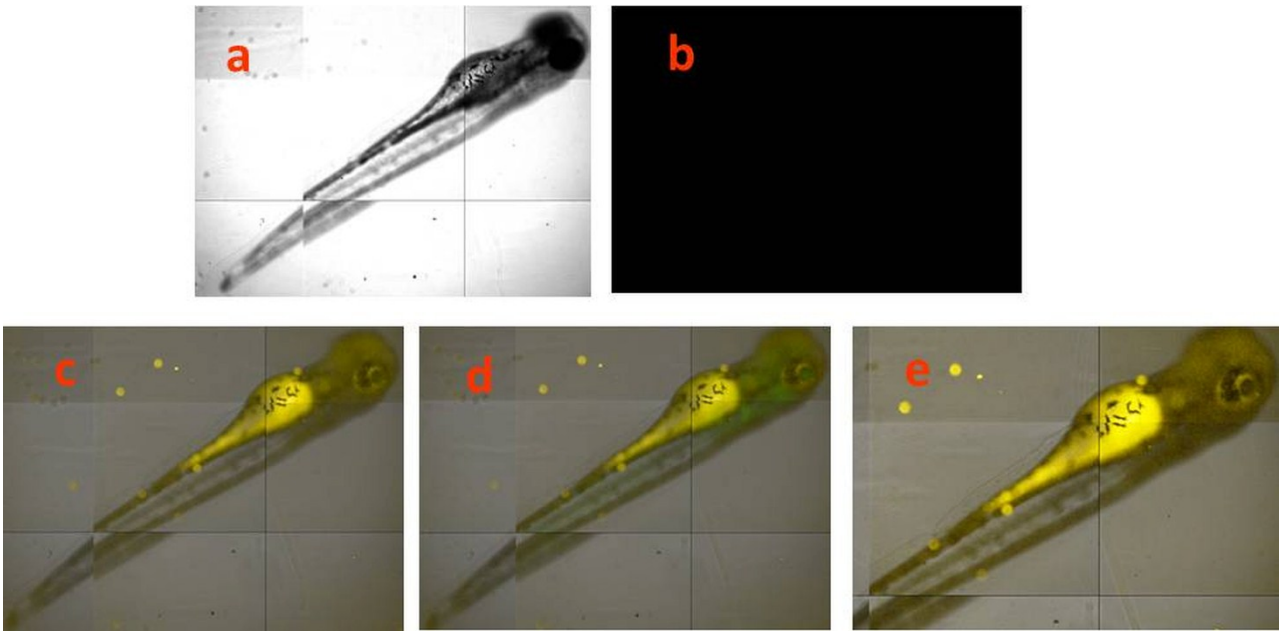



**Fig-S17: TryptA fluorescence imaging analysis in 4 days old zebrafish embryos fed with different concentrations of TryptA (a) bright field images of pre-treated TryptA (50 µM), (b) fluorescence merged images of pre-treated TryptA (c) 10 µM of TryptA (d) 25 µM of TryptA (e) 50 µM of TryptA for 2 h followed by incubation with PYNA (50 µM) for 1 h.**

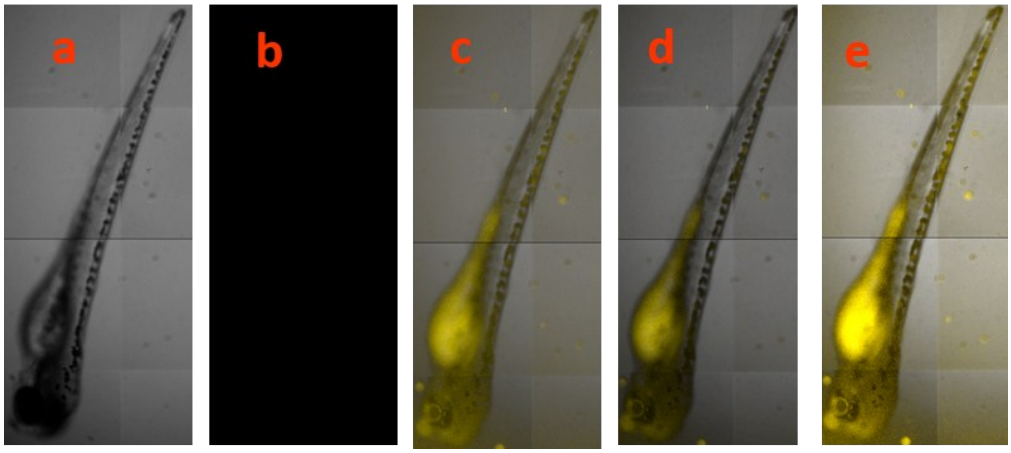



**Fig-S18: $F^-$ ions fluorescence imaging analysis in 4 days old zebrafish embryos fed with different concentrations of $F^-$ ions (a) bright field images of pre-treated $F^-$ ions (50 µM), (b) fluorescence merged images of pre-treated TryptA (c) 10 µM of $F^-$ ions (d) 25 µM of $F^-$ ions (e) 50 µM of $F^-$ ions for 2 h followed by incubation with PYNA (50 µM) for 1 h.**

**Table S1:Previous reports of Tryptamine receptors and their LOD:**

| S.No | Receptors | Fluorescence Responses | LOD | Applications | References |
|---|---|---|---|---|---|
| 1 | Nanofibre mat derivative | Turn - On | 6 $ngmL^{-1}$ | ------ | T.R.Marquez, et al., *Biosensors and Bioelectronics.,* **2016**,(79), 600–607 |
| 2 | Appending zinc tetraphenylporphyrin with an amine receptor at b-pyrrolic carbon for designing a selective histamine chemosensor | Ratiometric | 20 μM | ------- | W.Liu., et al., *Org. Lett.,* Vol. 9, No. 19, **2007** |
| 3 | Zinc(II) protoporphyrin as a functional monomer | Turn - On | 0.1–1 mM | ------- | Bao, et al., *Anal. Chem.* **2002**, (74), 1144–1148 |
| 4 | Calcein-ligand exchange mechanism | Turn - On | 0.5 μM | ------- | D.Seto., et al. *Bioorg. Med. Chem. Lett.*, **2012** (22) 4014–4017 |
| 5 | Comparison of Different Enzyme Electrodes : | Turn - On | 5 μM | ------ | B. Boka.,et.al, *Electroanalysis* **2012**, (24), 181 – 186 |
| 6 | Montmorillonite-Supramolecular Hydrogel Hybrid | Turn - On | 50 μM, | | Z. Sun, et.al, *Chem. Pharm. Bull.* **2016**, (64),1 |
| 7 | Molecular Recognition and Discrimination of Amines with a Colorimetric Array | | 60 ppm | ------ | N.A.Rakow., et.al, *Angew. Chem.* **2005**, (117), 4604 – 4608 |
| 8 | A molecular probe for the optical detection of biogenic aminesw | Turn - On | 25 μM | ------ | B.Lee., et.al, *Chem. Commun*., **2011**,(47), 9639–9641 9639 |
| 9 | Sensory hybrid host materials for the selective chromo-fluorogenic detection of biogenic amines | Turn - On | $5\times10^{-4}$ M | ------ | B.G.Acosta., et.al, Chem. Commun., **2006**, 2239–2241 |
| 10 | This work | Ratiometric | $0.4\times10^{-10}$ M | Live cell imaging | |

**Table S2: Previous reports of $F^-$ ion receptors and their LOD**:

| S.No | Receptors | Fluorescence Responses | LOD | Applications | References |
|---|---|---|---|---|---|
| 1 | Fluorene derivative | Turn-On | $1 \times 10^{-4}$ M | ------ | B.Sui, et al. J. *ACS Appl. mater. Interfaces*, **2013**, (5), 2920−2923 |
| 2 | Pyrene-1-carboxaldehyde hydrazone derivative | Colorimetric responses | $1.7 \times 10^{-6}$M | ------ | S.Ghosh, et al., *Bull. Korean Dalton Trans.,* **2016**, (45), 11042–11051 |
| 3 | N-phenylthiosemicarbazide derivatives | Turn - On | $0.18 \times 10^{-8}$M | ------- | S.velmathi et al., *Sensors and Actuators B*, **2014,** (204) 204375–381 |
| 4 | Diketopyrrolopyrrole derivative | Turn - On | $1\times 10^{-5}$ M. | ------- | X.yang, et al., *Sensors and Actuators B,* **2015** (207) 9–24 |
| 5 | Coumarin derivative | Turn - On | 0.2 mg/L | ------- | R.Chavali, et al. *Analytical chemistry Research,* **2015** (6) 26e31 |
| 6 | 1,8-diaminonaphthalene derivatives | Colorimetric responses | $2 \times 10^{-5}$ M | ------ | K.C. Nam, et.al, *Org.Lett.,* **2005** (7), 2607-2609 |
| 7 | Amino-functionalized metal-organic framework | Turn - On | 1600µg | Live cell imaging | F.M. Hinterholzinger, et.al, *Scientific reports* \| 3 : 2562 \| DOI: 10.1038/srep02562 |
| 8 | Bis-ureidoquinoline derivative | Turn - On | $1.0 \times 10^{-5}$ M | ------ | Y. Jo, et.al, *J. Org. Chem.* **2014**,(79), 9418−9422 |
| 9 | Azo-benzimidazole derivative | Turn - On | $9.189 \times 10^{-8}$ M | ------ | D.Chellappa, et.al, *Journal of Luminescence,* **2015,** (157) 383–389 |
| 10 | This work | Ratiometric | 0.30 nm | Live cell imaging | |

**References**

1. A. Senthil Murugan, N. Vidhyalakshmi, U. Ramesh and J. Annaraj *J. Mater. Chem. B,* **2017**, 5, 3195-3200

2. S. Goswami, D. Sena , N. K. Dasa , H-K Funb and C. K. Quah *Chem. Commun.*, **2011**,47, 9101.

3. R. Imran Khan and K. Pitchumani. *RSC Adv.,* **2016**, 6, 20269-20275