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Synthesis and photophysical properties of water soluble phosphonic acid functionalized naphthalimide dye

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In this work, a water-soluble phosphonate containing chromophore has been designed and synthesised. The prepared compound phosphonate-naphthalimide-morpholine (**NPI-M-P2**) has been investigated for its photophysical characteristics by means of UV-Vis and fluorescence spectroscopy at different *p*H ranging from 2 to 10. Upon excitation of absorption band at 410 nm, **NPI-M-P2** exhibits strong fluorescence emission peak maxima at 565 nm. With the increase in *p*H from 3 to 9, emisison peak intensity of **NPI-M-P2** increases and displays a more intense green color.

Keywords: Naphthalimide, Phosphonic acid, Morpholine, Optical property, Fluorescence emission

In the living organisms, phosphonate groups are ubiquitous and are an integral part of biogeochemical phosphorus cycle[1,2]. Phosphonate compounds exhibited bioactive properties and World Health Organization (WHO) recognized fosfomycin[3], tenofovir[4] and zoledronic acid[5] as essential medicines. Moreover, *N*-phosphonomethylglycine (glyphosate) and naturally occurring phosphinate phosphinothricin (glufosinate) are utilized as efficient herbicides and capture more than 30% of the global market[6]. The C-P bonds of phosphonates are strong enough to resists enzymatic degradation in natural systems. Therefore, phosphonates target the more crucial activities of cellular function. It is also well documented that phosphonates can phosphorylate enzyme hydrolase catalytic sites resulting in covalently bound phosphonic esters[7]. Phosphonates can interact with the enzymes *via* non-covalent interactions such as strong electrostatic interactions with positively charged amino acid residues present in the enzymes[8]. Moreover, the phosphonate moiety exhibits pK_a of ~ 7.6 indicating that these functional groups remain ionized[9] at physiological *p*H. These properties make phosphonate functional groups more attractive for constructing water soluble and stable chromophores[10]. The organic fluorescent probes overcome the issue of insolubility in water. Such water-soluble fluorophores are important for real-time monitoring of the living objects in biological systems[11].

Herein, we developed new naphthalimide based fluorophore (**NPI-M-P2**) by attaching donor (D) morpholine (**M**) moiety at aromatic core and water soluble (3-aminobenzyl)phosphonic acid subunit (**P2**) at imide position as depicted in Fig. 1. The complementary subunit naphthalimides acts as a fluorophore and it has high photo/thermal/chemical stability and fluorescence quantum yield in dilute solution[12]. Moreover, naphthalimide ring attached functional groups at 3 and 4 position has beneficial to manipulate photophysical properties and target ability of bio-molecules[13,14]. The third subunit the morpholine, which exhibits donor properties, is incorporated in the molecular structure of **NPI-M-P2**[15-17]. Phosphonate functional group was establishing to increase the solubility and stability of the chromophore in water[9-11]. The present investigation focused on the influence of morpholine on photophysical properties of **NPI-M-P2**. Such new generation **NPI-M-P2** chromophore have enormous potential in the area of fluorescent sensor and bioimaging applications[18].

† Both the authors contributed equally to the article.

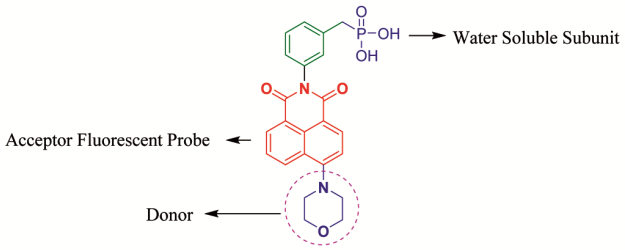


Fig. 1 — Molecular structure of the fluorophore **NPI-M-P2**

Experimental Section

Materials and Methods

Reagents and chemicals utilized in this work are 3-nitrobenzaldehyde, sodium borohydride ($NaBH_4$), phosphorus tribromide (PBr_3), triethyl phosphite [$P(OEt)_3$], 10% Pd/C, trimethylbromosilane, ethanol, methanol, acetic acid, tetrahydrofuran, potassium hydroxide, zinc acetate, dichloromethane. These chemicals were procured from Alfa Aesar Pvt. Ltd. India, TCI, India and Sigma-Aldrich Pvt. Ltd., India. Solvents were acquired from Finar Pvt. Ltd, India Spectrochem, India and Merck Pvt. Ltd., India. Prior to use, solvents were purified by the standard purification protocol.

^1H, ^{13}C and ^{31}P NMR spectra were measured at 300, 400 or 500 and 75, 100 or 125 MHz, respectively. Tetramethylsilane (TMS) was used as an internal standard. FT-IR experiments were performed using Thermo Nicolet Nexus 670 instrument. High-resolution mass spectra (HRMS) experiments were performed using Thermofisher Exactive Orbitrap on FTMS. UV-vis experiments were carried out using a UV-Vis-1800 Schimadzu spectrophotometer (Installed in CSIR-IICT, Hyderabad, India). Fluorescence emission spectras were recorded using RF-6000 (Schimadzu, Japan) spectrofluorophotometer (Installed in CSIR-IICT, Hyderabad, India).

Synthetic procedure for compound, 2

Compound **2** was prepared following literature protocol[19,20,] typically, in a 50 mL RB in which 3-nitrobenzaldehyde **1** (2.0 g, 13.23 mmol) in 30 mL dry methanol and sodium borohydride (0.567 g, 15.8 mL) was stirred for 5 minute and then after refluxed for 8 h. After completion of reaction, solvent was removed by rotary evaporator, cooled at RT. The obtained residue was dissolved in diethyl ether (500 mL), washed several times using saturated sodium bicarbonate followed by water and brine. The organic layer was passed through sodium sulphate, Solvent was removed and recrystallized in DCM-hexane, obtained **2** as analytical pure solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.23 (S, 1H), 8.13-8.11 (d, 1H, J = 8.08 Hz), 8.70 – 8.68 (d, 1H, J = 7.30 Hz), 7.54 – 7.50 (t, 1H), 4.80 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.3, 142.9, 132.7, 129.4, 122.4, 121.4, 63.8.

Synthetic procedure for compound, 3

A solution of phosphorus tribromide (1.4 mL 14.49 mmol) in 10 mL anhydrous dichloromethane was added slowly to the compound **2** (1.85 g 12.08 mmol) in 30 mL anhydrous dichloromethane at 0°C. Then, the reaction mass was stirred for further 1 h at 0°C and followed at RT for 12 h. Furthermore, the reaction mass was poured into crushed ice and extracted with diethyl ether (300 mL). The obtained organic phase was washed several times with saturated $NaHCO_3$ solution, brine and Na_2SO_4. Then, the solvent was removed and recrystallizes by DCM-hexane, which in turn yielded **3** as analytical pure solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.25 (1H), 8.15 (1H), 7.73 – 7.72 (d, 1H, J = 7.70 Hz), 4.54 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 148.3, 139.7, 135.0, 129.9, 123.9, 123.3, 30.8.

Synthetic procedure for diethyl 3-nitro-benzylphosphonate derivative, 5

First Step: In 50 mL clean oven dried sealed tube compound **3** (1.03 g 4.7 mmol) and triethyl phosphite (10 mL) was heated at 160°C for 12 h. After completion of reaction, the reaction mass was cooled. Then, the excess triethyl phosphite was evaporated under reduced pressure and the obtained residue was stirred in diethyl ether. The crude product was purified using column chromatography (DCM: MeOH = 100:2) to yield diethyl 3-nitrobenzylphosphonate **4** as thick oil. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.05 – 8.03 (d, 2H, J = 8.06 Hz), 7.85 – 7.82 (d, 2H, J = 8.06 Hz), 7.50 – 7.46 (m, 2H), 7.39 – 7.35 (m, 2H), 4.94 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.5, 153.0, 135.7, 128.2, 126.1, 125.2, 123.1, 121.5, 38.8.

Second Step: In a clean oven dried 50 mL RB flask, a solution of diethyl 3-nitrobenzylphosphonate **4** (0.83 g 3.0 mmol**)** and 10 mg of 10% Pd/C in 20 mL of ethanol was added and the reaction mixture was stirred at RT for 12 h under H_2 atmosphere. After completion of reaction (TLC), the reaction mass was filtered through a celite bed, washed with 30 mL of ethanol. The crude product was purified with flash chromatography (DCM:MeOH = 95:5) to yield

compound **5**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05–8.03 (d, 2H, *J* = 8.06 Hz), 7.85–7.82 (d, 2H, *J* = 8.06 Hz), 7.50 – 7.46 (m, 2H), 7.39–7.35 (m, 2H), 4.94 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 146.7, 132.4, 129.3, 119.8, 116.3,113.7,62.1, 34.3,33.0,16.4.

Synthetic procedure for compound, 6

1,8-naphthalicanhydride (NPA) (4.0 g 20.184 mmol) was added to 25 mL of 4 M aqueous potassium hydroxide (KOH) in a clean oven dried RB flask and purged with nitrogen. The reaction mixture was cooled to 0°C. Then bromine (2.01 mL 40.369 mmol) was added drop-wise to the reaction mixture up to 2 h. After completion of bromine addition, the reaction mass was heated at reflux temperature for 20 h. After completion of reaction (confirm by TLC), the reaction mixture was cooled to RT. Then acidified with 10 mL concentrated H_2SO_4 and further refluxed for another 2 h. Then it was cooled to RT, then the solution was poured into crushed ice and filtered it. The residue was washed with cold water three times, dried it in oven and compound **6** was further used for next step. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.64-8.60 (t, 2H), 8.38-8.36 (d, 1H, *J* = 7.88 Hz), 8.28-8.26 (d, 1H, *J* = 7.88 Hz), 8.07-8.03(t, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.7, 134.1, 133.7, 133, 132.1, 131.0, 130.7, 130.4, 129.6.

Synthesis of diethyl(3-(6-bromo-1,3-dioxo-1*H*-benzo-[de]isoquinolin-2(3*H*)-yl)benzyl) phosphonate (NPI-Br)

Compound **5** (0.526 mg 2.16 mmol) and compound **6** (500 mg, 0.2113 mmol) were added in 15 mL dry ethanol in a 100 mL sealed tube. The reaction mixture was stirred for 5 minutes. then zinc acetate (30 mg 2.16 mmol) was added as an additive, and the reaction mixture was further heated at reflux temperature for 24 h. After completion of reaction (confirmed by TLC), the reaction mass was cooled slowly to RT. The reaction mixture was poured in ice. The precipitate was filtered and the obtained residue was washed with cold water. Then it was dried in vacuum oven. The crude buff white solid was purified by column chromatography on silica gel (100-200 mesh) using DCM: MeOH (100:2, volume ratio) as an eluent to afford **NPI-Br**. (0.298 mg, 63.26%). FT-IR (KBr): 2921, 2851, 2213, 1705, 1665, 1572, 1428, 1376, 1310, 1251, 1185, 1086, 973, 935, 799, 756, 722, 586, 546 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.70-8.68 (dd,1H), 8.46-8.44 (dd, 1H), 8.10–8.08 (d, 1H), 7.91-7.87 (t, 1H), 7.53–7.44 (m, 2H), 7.26 (s, 1H), 7.22–7.20 (dd, 2H), 4.08–4.00 (m, 4H),3.26–3.21(s, 2H), 1.28–1.22 (t, 6H), 0.86–0.84 (t, 6H); ^{13}C NMR ($CDCl_3$,100 MHz): δ 163.6, 133.6, 132.4, 131.5, 131.2, 130.7, 130.2, 130.0, 130.0, 129.5, 128.2, 127.1, 122.3, 62.5, 34.4, 33.0, 16.4, 16.4; MS(ESI$^+$): *m/z* Calcd for $C_{23}H_{21}BrNO_5P$:502.30 $[M]^+$. Found: 502.04 $[M]^+$.

Synthesis of diethyl (3-(6-morpholino-1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)benzyl) phosphonate (NPI-M-P1)

Diethyl(3-(6-bromo-1,3-dioxo-1*H*-benzo[de] isoquinolin-2(3*H*)-yl)benzyl)phosphonate (**NPI-Br**) (298 mg, 0.587 mmol and morpholine (0.07 mL 0.880 mmol) were taken in an oven dried RB flask. Dry THF (10 mL) was added to the flask and purged with nitrogen for 15 minutes. The reaction mixture was stirred at RT for 15 minutes, followed by stirred at 70°C for 24 h. The progress of reaction was monitored with TLC. After completion of reaction, the reaction mass was cooled to RT. Then the solvent was evaporated under reduced pressure using rotary evaporator. The obtained crude product was purified by silica gel (100–200 mesh) column chromatography using DCM:MeOH (100:5, volume ratio) as an eluent to afford yellowish solid of **NPI-M-P1** (0.298 mg, 89.23%). FT-IR (KBr): 3449, 3068, 2080, 2900, 2846, 1699, 1659, 1585, 1514, 1370, 1246, 1246, 1192, 1120, 1028, 962, 910, 782, 695, 617, 503 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.63-8.55 (dd, 2H), 8.49-8.47 (d, 1H), 7.77 – 7.72 (t, 1H), 7.52 – 7.43 (m, 2H),7.27 (s,2H),7.22-7.20 (d,1H), 4.06-3.99 (m,8H), 3.30-3.27 (t,4H),3.20(s,2H)1.28-1.24(t,3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.1, 164.0, 155.9, 135.8,132.9, 131.9, 130.4, 130.1, 129.4, 127.3, 126.3, 125.9, 123.5, 117.2, 115.0, 67.0, 62.4, 53.4, 34.4, 33.1, 16.4.; HRMS: Calcd for $C_{27}H_{29}PN_2O_6$: *m/z* 508.51 $[M]^+$. Found: 526.2139 $[M+NH_4]^+$.

Synthesis of (3-(6-morpholino-1,3-dioxo-1*H*-benzo [de]isoquinolin-2(3*H*)-yl)benzyl) phosphonic acid (NPI-M-P2)

Diethyl (3-(6-morpholino-1,3-dioxo-1H-benzo[de] isoquinolin-2(3H)-yl)benzyl)phosphonate (**NPI-M-P1**) (0.298 g 0.588 mmol) was taken in an RB flask. Under inert atmosphere, 10 mL dry dichloromethane was added to the flask and cooled to 0°C. TMSBr (0.310 mL, 2.30 mmol) was added dropwise to above cooled solution with stirring. After completion of addition of TMSBr, the ice bath was removed. Then the reaction mixture was stirred at RT for 1 h, followed by further stirring at 50$^{°C}$ for 8 h. The

progress of reaction was monitored using TLC. After completion of reaction, the reaction mixture was cooled to RT. Then the volatiles were removed under vacuum. Furthermore, the crude product was suspended in 95% methanol and stirred overnight. The obtained precipitated solid was filtered, washed with methanol and dried in oven. The crude product was purified using column chromatography on silica gel (100–200 mesh) DCM: MeOH (100:10, volume ratio) as an eluent to afford yellowish solid of **NPI-M-P2** (0.240 g, 90.23%). FT-IR (KBr): 3420, 2958, 2022, 2852, 1702, 1858, 1587, 1514, 1451, 1371, 1237, 1116, 1044, 1007,908, 848, 785, 752, 694, 621, 502, 411 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz, T= 60°C): δ 8.51-8.39 (m, 3H), 7.81 (s,1H), 7.37 (m,3H), 7.19– 7.16 (d, 1H), 4.81 (2H), 3.91 (s, 4H),3.24 (s, 4H), 3.14-3.00(dd, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz, T= 60°C): δ 164.2, 163.7, 156.0, 136.3, 135.7,135.6, 134.8, 134.7, 132.7, 131.2, 130.0, 129.0, 128.8, 127.5, 126.6, 125.7, 123.5, 116.7, 115.5, 66.6, 61.2, 61.1, 53.5, 36.3, 34.9, 34.5, 33.1, 16.8; ^{31}P NMR (DMSO-d_6,T= 60°C): δ 25.78; HRMS Calcd for $C_{23}H_{22}PN_2O_6$: *m/z* 453.1210; Found: 453.1220 $[M+H]^+$.

Results and Discussion

Design of NPI-M-P2

The proposed **NPI-M-P2** dye was designed based on naphthalimide fluorescent probe (Fig. 2). We chose NPI as core fluorophore due to their photophysical properties and chemical as well as thermal stability with significant fluorescence quantum yields. The aromatic NPI core and its imide N site can be altered through organic synthetic strategy. It is well documented that NPI core is an electron acceptor moiety. The NPI fluorophore probe is modified with morpholine moiety at aromatic core and (3-aminobenzyl)phosphonic acid unit at imide position. The morpholine subunit is attached due to its high pK_a values (~8) and acts as donor entity. We select (3-aminobenzyl)phosphonic acid unit to modify imide position of NPI because phosphonic acid is a functional group bearing two –OH moieties, one P=O bond and one more P-C bond. Phosphonic acid functional group was incorporated in the molecular architecture to introduce specific properties such as water solubility, chelating and/or supramolecular properties. Moreover, phosphonic acid is a subunit that is readily involved in non-covalent hydrogen bonding. The third important property of phosphonic acid arises from its three oxygen atoms, which can be involved in iono-covalent bond. Thus, we presume that the designed fluorophore **NPI-M-P2** with excellent water solubility, fluorescence properties and high photostability can exhibits pronounced sensitivity for future bioimaging applications.

Synthesis of chromophore NPI-M-P2

NPI-M-P2 is synthesized according to the route depicted in Scheme 1. At first, synthesis of (3-aminobenzyl) phosphonic acid **5** was achieved starting from 3-nitrobenzaldehyde **1** in four steps using modified procedures[19,20]. Aniline **5** was reacted with 4-bromo-1,8-naphthalic anhydride **6** in ethanol/zinc acetate to afford compound **NPI-Br** with 63% yields. The subsequent reaction of NPI-Br with morpholine in dry THF gave compound **NPI-M-P1** with 89% yields[21]. At last, the hydrolysis of phosphonate ester **NPI-M-P1** was achieved in the presence of tetramethylsilyl bromide (TMSBr) affording **NPI-M-P2** in 90% yields. The molecular structure of **NPI-M-P2** was fully characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P spectra and HRMS spectrum (See the Supporting Information). The spectroscopic study agreed well with the expected structure of **NPI-M-P2**.

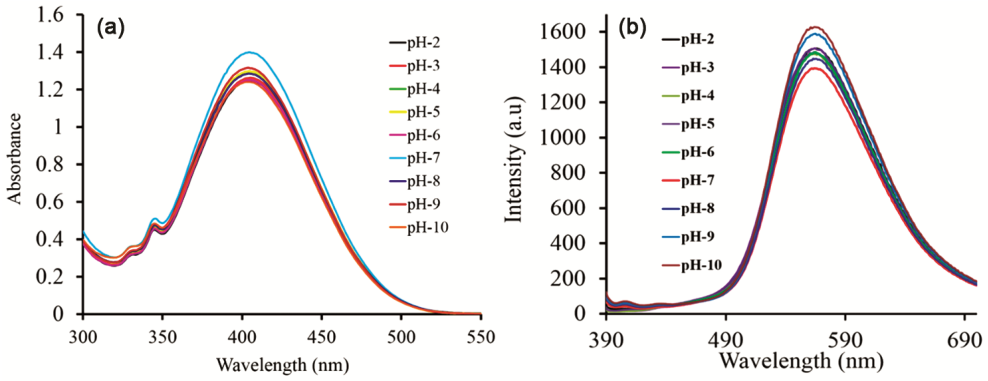
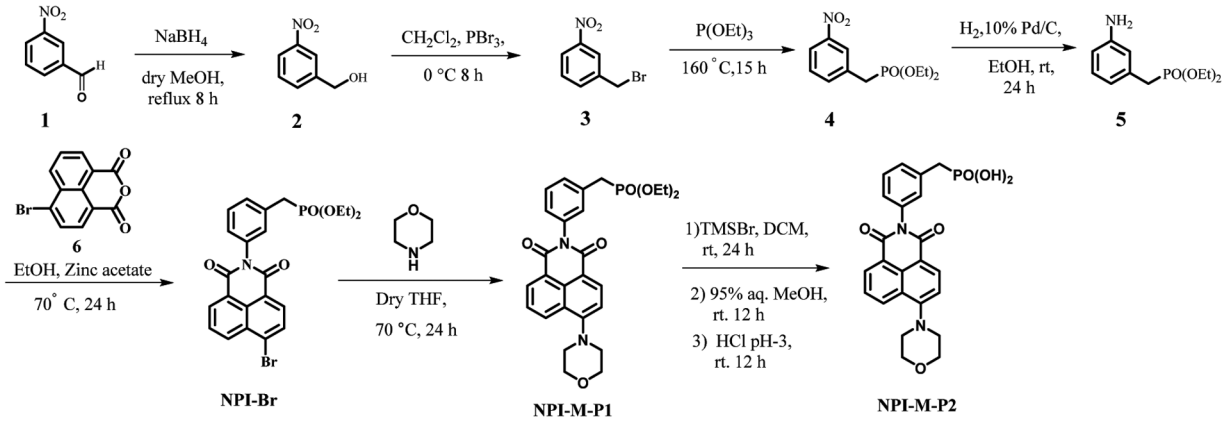


Fig. 2 — (a) UV-Vis spectra and (b) fluorescence spectra (λ_{ex} = 410 nm) of **NPI-M-P2** (1×10^{-4} M) in DMSO with BR buffer of *p*H 2-10



Scheme 1 — Synthesis of **NPI-M-P2**

The UV-Vis and fluorescence emission spectra of **NPI-M-P2** properties were studied in BR buffer solution (1×10^{-4} M) with different *p*H values. The results for UV-Vis and fluorescence characteristics are depicted in Fig. 2a and Fig. 2b, respectively. As shown in Fig. 2a, the UV-Vis absorption maxima of **NPI-M-P2** at *p*H 2 appeared at 406 nm. The change is *p*H of solution from 2 to 10, display negligible changes in absorption intensity. As shown in Fig. 2b, the excitation (λ_{ex} = 410 nm) of **NPI-M-P2** probe at *p*H 2 exhibit emission maxima at 565 nm, indicating the emissive nature of **NPI-M-P2**. Upon changing *p*H from 2 to 10, increase in intensity of emissive band was observed. From these studies, it is clear that probe **NPI-M-P2** is highly fluorescent at different *p*H varying from 2 to 10.

Furthermore, we also examined the fluorescence emission changes of **NPI-M-P2** in methanol at different *p*H and the results are depicted in Fig. 3. At *p*H 3, the emission peak intensity was lowered as compare to *p*H 7 and 9. It was found that at *p*H 9, the fluorescence emission intensity is higher. It is also confirmed by naked-eye fluorescent color change of the **NPI-M-P2** solution (Fig. 3b). At *p*H 9, the solution color is highly emissive green compare to *p*H 3 (Fig. 3b). It indicates that upon protonation the photoinduced electron transfer (PET) effect is observed[16]. Thus, PET is off at *p*H 9 whereas, on at *p*H 3. The nitrogen atom of morpholine is in conjugation with NPI, at *p*H 3, upon protonation the complete NPI system become electron deficient and the photoinduced electron transfer takes place from morpholine oxygen to the NPI core, attributed to decrease in emission intensity. **NPI-M-P2** material is interesting to investigate its application in medicinal and bioimaging fields. Work in this direction is in progress and will report in due course of time.

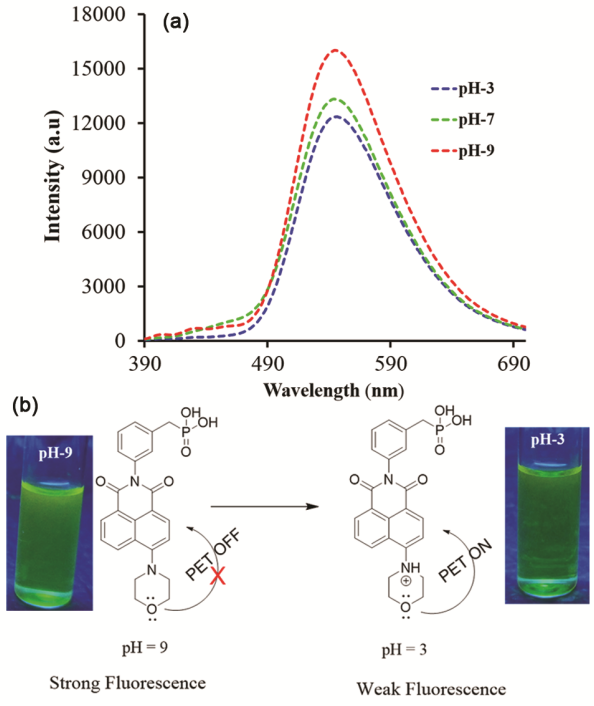


Fig. 3 — (a) Fluorescence emission spectra of **NPI-M-P2** in MeOH at *p*H 3 (blue dotted line), 7 (green dotted line) and 9 (red dotted line) [data extracted and zoomed plot are shown from Fig. 2b] and (b) PET 'off-on' mechanism and naked eye color change of the **NPI-M-P2** solutions

Conclusions

We have developed **NPI-M-P2** chromophore from naphthalimide. NPI was conjugated with morpholine. The water-soluble phosphonate group was incorporated at imide position. The morpholine receptor in

conjugation with NPI displayed the PET process. The morpholine core responded to change in *p*H ranging from 2 to 10 and tuned the photophysical and optical properties of NPI fluorophore. The present investigation leads to develop novel water-soluble chromophore with potential applications in medicinal as well as bioimaging applications.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with Ethical Standards

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/ 58776

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