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Fluorescent azophenol-quinazoline dyad as multichannel reversible pH indicator in aqueous media: an innovative concept on diazo based dyads

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ABSTRACT

A novel azophenol-quinazoline dyad **1** has been designed, synthesized and demonstrated as an efficient reversible multichannel pH indicator through distinct signalling in aqueous media. Owing to the competence between highly fluorescent quinazoline moiety and a well known fluorescence quencher diazo group, dyad **1** is moderately fluorescent in nature. Under acidic conditions **1** displays diverse fluorogenic changes (blue emission at pH 4.25; green at pH 1.80) while under basic condition (pH 11.80) chromogenic changes were observed.

The detection and control of pH play an important role in chemistry, biochemistry, cellular biology, drug delivery etc.[1,2] A general approach to perceive the pH changes principally relies on naked eye detection using pH indicators.[2c] Internal pH in various prokaryotes and cellular organelles of the eukaryotes can vary from basic to highly acidic values.[3] Though majority of the living species cannot survive under extremely acidic conditions[4] (pH <4), numerous microorganisms including acidophiles, helicobacter pylori, enteric pathogens etc. prevail under such harsh conditions.[3b] Measuring the internal pH of living cells, mostly the lower ones is a challenging task. In this context, development of pH indicators stable below pH ~4 and capable of detecting extremely low and high pH, especially via multichannel signalling are highly demanding.

Considering the limitations of pH detection in biological systems through chromogenic responses only, attempts are being made to develop highly sensitive fluorescent pH indicators with distinct signalling.[1,2] Measurement of the physiological pH through fluorescence performance offers many advantages over other detection methods due to high sensitivity and selectivity of this technique.[5] Han and Burgess reported many fluorescent dyes derived from fluorescein, benzoxanthene, cyanine, rhodols, acridine and BODIPY dyes as a probe for the determination of pH.[6] Stability of these pH indicating dyes in a high to low pH is always questionable, however HdeA58-DMN and HdeA35-DMN have been used as low pH (~2.0) and FAM35 as high pH (~11.0) indicator through fluorescence enhancement.[3b,7] Though, many chromogenic and fluorogenic pH indicators have been tested under acidic and basic conditions, those working as a pH indicator for highly acidic and basic conditions are still indispensable. Furthermore, molecules capable of detecting multiple pH through multichannel techniques and distinct signalling in practice are completely lacking.

Quinazolines are familiar biologically important compounds as antagonist, DNA-biosensor, epidermal growth factor receptor tyrosine kinase (EGFR-TK) imaging, activin-like kinase (ALK5) inhibitors etc.[8] Recently, we have shown that these are highly fluorescent in nature and have used as fluorescent probes for the detection of heavy and transition metal ions.[9] In addition, diazo compounds are extremely useful as dyes and pigments[10] owing to their intense colour and some of these exhibit colour change with pH.[11] The fluorescence quenching in these systems arising from picosecond photoswitching[12,13] as well as photoinduced electron transfer (PET)[14] in the diazo group limits their application as fluorescent pH indicators. Therefore, it could be interesting to combine quinazoline fluorophores with diazo chromophore to develop an elegant dyad system. However, Tamaoki co-workers and Ishow co-workers have tactfully designed and developed fluorescent azo dyads by combining a well known fluorophore unit with the diazo group. They suggested that to develop a fluorescent azo dyad, there must be a good separation between the absorption and emission bands of the fluorophore from that of the diazo chromophore to

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avoid spectral overlap.[13] Considering the absorption of quinazolines (~350 nm)[9] which is in the range of diazo absorptions (~330–410 nm),[15a] we were curious to examine the competitive effect on emission of a dyad resulting from strong fluorescence of quinazoline fluorophore and inherent quenching of the diazo group. It seemed quite challenging since the theme is not in keeping with the concept of Tamaoki co-workers and Ishow co-workers.

Our prime concern to execute this work was to have a deep insight of the aforesaid concept for azo dyads by developing an azophenol-quinazoline dyad [4-(5,6-dihydrobenzo[4,5]imidazo-[1,2-*c*]quinazolin-6-yl)-2-(4-nitrophenylazo)phenol (**1**)]. Notably, our anticipation turned up positively as **1** is moderately fluorescent and acts as a multichannel (chromogenic and fluorogenic) reversible pH indicator with distinct signalling under highly acidic as well as basic conditions in aqueous media. The present system is not only a particular pH indicator through colorimetric responses, but also a well designed azo based dyad, exhibiting conceptually challenging emission behaviour and detects various pH (11.80, 7.10, 4.25 and 1.80) through multichannel (chromogenic and fluorogenic) pathway and distinct signalling. This approach makes the system unique relative to other available pH indicators and to our knowledge it is the first report dealing with an amendment in the concept about azo based dyads and development of a system capable of detecting multiple pH.

The dyad **1** was synthesized by condensation of 4-hydroxy-3-(4-nitrophenylazo)-benzaldehyde (**2**) with 2-(2-aminophenyl)-1-benzimidazole in ethanol (Scheme S1, Supporting information).[9] It has been completely characterized by satisfactory elemental analyses and spectral (FT-IR, ^{1}H and ^{13}C NMR, ESI-MS, UV/vis and emission) techniques (Figs. S1–S5). The phenolic –OH proton of **1** appeared as a singlet at δ 11.07 ppm in its ^{1}H NMR spectrum (dmso-d_6), while quinazoline –NH (*Hg*) resonated as a singlet at δ 7.82 ppm. The formation of quinazoline is further evidenced by the presence of another singlet at δ 7.08 ppm due to –CH (*Hf*) proton of the quinazoline ring. Two sets of nitrobenzene ring protons (*Ha*/*Ha*′–*Hb*/*Hb*′) resonated in the downfield side as doublets at δ 8.42 and 8.15 ppm. Other aromatic protons appeared in the range of δ 7.98–6.83 ppm (Fig. S4). Further, an intense peak at *m*/*z* 463.1646 $[M+H]^+$ in the ESI-mass spectrum of **1** strongly supported formation of this compound (Fig. 1b).

The electronic absorption spectrum of **1** (EtOH/H_2O; 1:99, 4 μM, pH ~7.10) exhibited a weak low energy (LE) band at 416 nm (ε, 1.3×10^4 M^{-1} cm^{-1}) due to $n-\pi^*$ transitions. On the other hand $\pi-\pi^*$ charge transfer transitions associated with azo moiety of **1** were displayed in the high energy (HE) side at 340 (ε, 3.2×10^4 M^{-1} cm^{-1}) and 304 nm (ε, 3.0×10^4 M^{-1} cm^{-1}) (Fig. 1a). To gain deep insight into the effect of pH on optical properties of **1**, UV/vis absorption titration experiments were performed by varying the pH to acidic as well as more basic (initial pH ~7.10, **1**) using aqueous solutions of 0.1 M HCl and KOH (Fig. 2a). Lowering the pH to ~4.25 leads to hyperchromic shift for both LE and HE bands along with a blue shift of 3 nm (Fig. 2b) for HE band to appear at 337 nm (ε, 3.7×10^4 M^{-1} cm^{-1}) which may arise due to

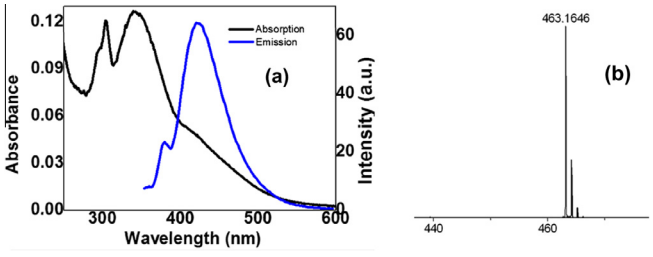


Figure 1. Absorption (black) and emission spectra (blue) of **1** (a), and ESI-MS of **1** showing molecular ion peak $[M+H]^+$ at *m*/*z* 463.1646 (b).

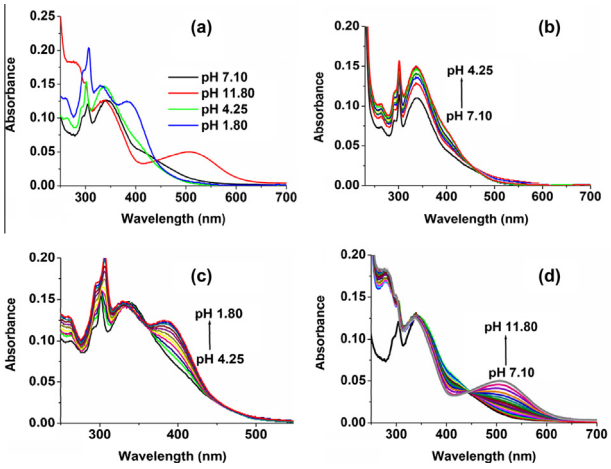
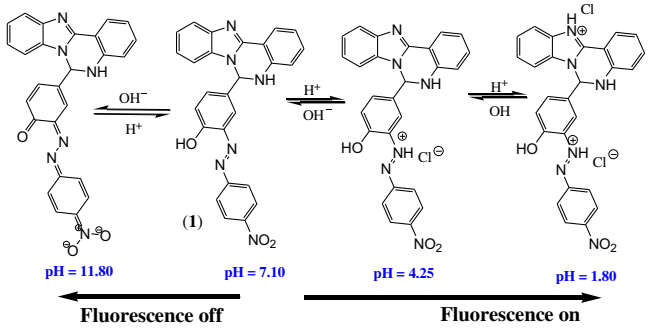


Figure 2. UV/vis spectra of **1** at pH 7.10 (black line), 11.80 (red line), 4.25 (green line), 1.80 (blue line) (a); UV/vis titration plot of **1** between pH 7.10 and 4.25 (b); pH 4.25–1.80 (c); pH 7.10–11.80 (d).

preferential protonation of the azo nitrogen (vide-supra). Further lowering of the pH to more acidic (pH ~1.80) causes a significant blue shift for both LE and HE bands to appear at 383 (ε, 3.2×10^4 - M^{-1} cm^{-1}) and 334 nm (ε, 3.7×10^4 M^{-1} cm^{-1}) with the emergence of an isosbestic point at 360 nm (Fig. 2c). At this pH, absorbance of the LE band increases significantly while that of the HE band remains unaltered. Blue shifting of the intense LE band at 383 nm may be attributed to the formation of quinazolinium ion (Scheme 1). It was observed that lowering the pH causes preferential protonation of the azo nitrogen due to its high electron density relative to quinazoline nitrogen.[13b] Dark yellow colour of the solution of **1** (pH ~7.10) turns light yellow and green yellow at pH 4.25 and 1.80, respectively (Fig. 3c).

In contrary, increasing the pH of a solution of **1** by addition of 0.1 M KOH leads to emergence of a new band at 509 nm while HE band exhibited a small blue shift (Fig. 2a). Further increase in basicity of the solution induces successive hyperchromic shift of the band at 509 nm and attains maximum value at pH ~11.80 (ε, 1.3×10^4 M^{-1} cm^{-1}). The HE band also displays a small blue shift of 4 nm and at pH ~11.80 appeared at 336 nm (ε, 3.2×10^4 M^{-1} - cm^{-1}). An increase in the pH from 7.10 to 11.80 results in isosbestic points at 445 nm (Fig. 2d). At this pH, colour of the solution turned dark pink red from dark yellow (Fig. 3c) and further addition of the base leads to insignificant changes in the spectral features. The changes under acidic and basic conditions in the UV/vis spectrum of **1** are completely reversible and attain almost the original band structure upon maintaining the initial pH ~7.10 (Fig. S6).

Compound **1** (λ_{ex}, 340 nm) shows moderate fluorescence at 430 nm [QY Φ; 0.05; Stokes shift (SS), 90 nm] (Fig. 1a). To



Scheme 1. Schematic representation of **1** showing complete reversibility under acidic and basic conditions.

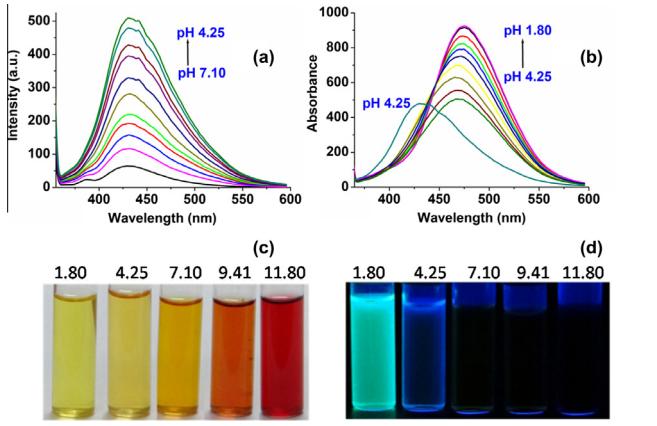


Figure 3. Fluorescence titration spectra of **1** in between pH ~7.10 and 4.25 (a), and pH 4.25–1.80 (b) for **1**, visible light (c), and fluorescence (d) images between pH 1.80 and 11.80.

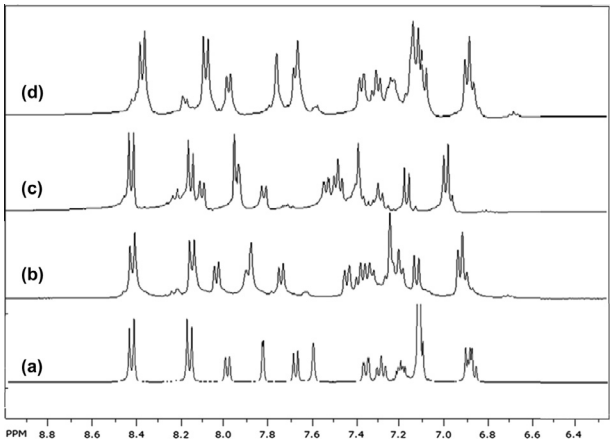


Figure 4. ^{1}H NMR titration plot for **1** (DMSO-d_6) under acidic pH upon addition of 0.1 M HCl [initial pH (a), pH ~4.25 (b), pH ~2.00 (c)] and regeneration of original spectra after adding 0.1 M KOH (d).

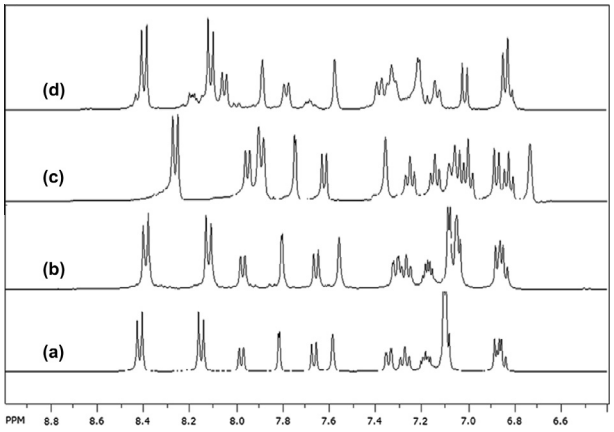


Figure 5. ^{1}H NMR titration plot for **1** (DMSO-d_6) under basic pH upon addition of 0.1 M KOH [initial pH (a), pH ~8.72 (b), pH ~12.00 (c)] and regeneration of original spectra after adding 0.1 M HCl (d).

investigate the effect of pH on stability and fluorescence behaviour of **1**, titration studies were performed by addition of an aqueous solution of 0.1 M HCl and KOH followed by proper mixing (Fig. S7). Lowering the pH to 4.25 from 7.10 causes a significant fluorescence enhancement (87%, eight fold) without any noticeable shift in the position of band (Fig. 3a). Almost six fold increase in the fluorescence QY at pH 4.25 (Φ; 0.28) may be ascribed to protonation of the azo nitrogen to form diazonium cation (Scheme 1). Further decrease in the pH of **1** displays an appreciable (fifteen fold) fluorescence enhancement [(475 nm; 93%) at pH 1.80 with a significant red shift of 45 nm (Fig. 3b)] The QY (Φ) at pH ~4.25 and ~1.80 was found to be 0.28 and 0.56 with respect to initial value. At pH 4.25 the dyad **1** shows a strong blue emission while at 1.80 it turns green (Fig. 3d).

Notably, based on our results from both the UV/vis and fluorescence studies, pH 4.25 can be taken as a critical pH point at which dyad **1** shows blue emission at 430 nm, and below this a green emission at 475 nm with significant red shift (45 nm). Fluorescence enhancement under acidic conditions may be associated with deactivation of the nitrogen lone pairs from the azo group as well as the quinazoline ring due to the formation of the quaternary nitrogen upon protonation. It may also arise due to hindrance on the flipping of the diazo group in its protonated form. Preferential protonation of relatively electron rich azo core[13b] (pH 4.25) results in an increase in the fluorescence intensity; however position of the band remains unaltered (430 nm). Further, lowering the pH below 4.25 led to an increase in the fluorescence intensity and the signal at 475 nm red shifted by 45 nm. It may be attributed to the protonation of quinazoline nitrogen and suggested that pH 4.25 acts as critical pH point and dyad **1** as a pH indicator towards various organic and biological reactions. Interestingly, changes in the fluorescence spectra under acidic pH are completely reversible and attain the initial spectral features upon addition of a base to make the pH about 7.10 (Fig. S8). On the other hand, increasing the pH up to 11.80 does not significantly alter the fluorescence behaviour of **1** (Fig. S7). Reusability of the dyad has been examined thrice through UV/vis and fluorescence experiments and observed that the system exhibits great pH indicating consistency (Fig. S8).

To elucidate the mechanism of structural changes with variation of pH, ^{1}H NMR titration experiments (dmso-d_6) were performed both under acidic and basic conditions (pH range ~2.00–12.00 (Figs. 4 and 5). Two step protonation of **1** under acidic conditions can be clearly understood from an appreciable downfield shift of the azophenol ring proton *Hc* (pH 4.25) from the initial value. Conversely, lowering the pH below 2.00 causes greater downfield shift due to the quinazoline protons (*Ho*, *Hl* and *Hg*) relative to *Hc* (Fig. 4). At pH ~4.25 the signals associated with *Hc* shifted largely towards downfield side and resonated at 7.91 ppm ($\Delta\delta$, 0.33 ppm), while quinazoline –NH (*Hg*) and –CH (*Hf*) displayed small downfield shift and appeared at δ 7.88 and 7.10 ppm ($\Delta\delta$, 0.06 and 0.02 ppm). Notably, lowering the pH to ~2.00 resulted in large downfield shift for the –NH (*Hg*) and –CH (*Hf*) protons and appeared at δ 7.96 and 7.15 ppm ($\Delta\delta$, 0.08 and 0.05 ppm), whereas *Hc* displayed small downfield shift and resonated at 7.93 ppm ($\Delta\delta$ = 0.01 ppm). It suggested preferential protonation at diazo nitrogen attached to the phenolic ring followed by quinazoline nitrogen (Scheme 1 and Table 1). Original ^{1}H NMR spectrum of **1** regenerated upon bringing the pH to initial value.

Under basic conditions (pH ~7.10–12.00) resonance due to phenolic –OH disappeared, while rational upfield shifts were observed for the phenolic and nitrobenzene ring protons (*Ha*–*He*). It may be ascribed to an increase in the electron density on these rings (Fig. 5, Scheme 1, and Table 1). Upon deprotonation of the –O*H* proton, conjugation is altered throughout the azophenol unit,[15b] which is responsible for upfield shifts of the protons associated with phenolic and nitrobenzene rings, however insignificant upfield shift was observed for the quinazoline protons. Lowering the pH to its initial value completely restores the original spectral features of **1**, strongly suggesting the reversible nature of **1** (Fig. 5).

Through this work a novel azophenol-quinazoline dyad **1**, exhibiting significant fluorescence, despite presence of a fluorophore excitation band between low and high energy bands of diazo

Table 1
^{1}H NMR data of **1** at various pH

Protons	pH ~7.10 (δ in ppm)	pH ~4.25 (δ in ppm)	pH ~2.00 (δ in ppm)	pH ~12.00 (δ in ppm)
*H*a	8.42	8.42	8.42	8.25
*H*b	8.15	8.12	8.12	7.89
*H*c	7.58	7.91	7.92	7.36
*H*d	7.33	7.43	7.53	7.25
*H*e	7.27	7.35	7.51	7.14
*H*g	7.82	7.88	7.96	7.75
*H*i	7.18	7.33	7.45	7.06
*H*j, *H*k	6.83–6.88	6.91–6.93	6.97	6.87–6.08
*H*l	7.66	7.74	7.80	7.62
*H*o	7.98	8.04	8.09	7.94
*H*f	7.08	7.10	7.15	6.73
*H*h, *H*m, *H*n	7.09–7.10	7.10–7.24	7.14–7.37	6.98–7.06

chromophore has been reported. The dyad acts as a multichannel (colorimetric and fluorometric) reversible pH indicator with distinct signalling under highly acidic and basic conditions in aqueous media. The pH 4.25 has been noted as a critical pH point below and above which it displays chromogenic change and fluorescence 'turn-on' along with significant red shift. This is the first report of any azophenol based dyad that is stable under highly acidic and basic conditions and reversibly detects three pH ranges (pH 7.10–4.25, 4.25–1.80 and 7.10–11.80) through distinct colour changes and fluorescence signalling. Ultimately, we have been able to propose a new concept on diazo based dyads that incorporation of a suitable fluorophore with a diazo group can lead to the creation of a new fluorescent dyad with small to comparable quantum yield despite absorption and emission band overlapping.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.08.103.

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