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An ESIPT fluorescent dye based on HBI with high quantum yield and large Stokes shift for selective detection of Cys[†]

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We report 3-(4,5-diphenyl-1*H*-imidazol-2-yl)naphthalen-2-ol (**DPIN**) as an interesting luminescent material displaying ESIPT with a large Stokes shift of ~180 nm even in protic/polar solvents. Stable homo-dispersed nanoparticles formed by inter- and intramolecular H-bonds in aqueous media and the corresponding aggregation induced enhanced emission with a high quantum yield up to 0.45 were observed. Factors such as pH value and ions (cations and anions) showed a negligible effect on the fluorescence performance. A probe of 3-(4,5-diphenyl-1*H*-imidazol-2-yl)naphthalen-2-yl-acrylate (**DPIN-A**) based on this molecule was designed. The results revealed that it can be used for sensing of Cys with high selectivity and sensitivity.

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1. Introduction

Molecules with excited-state intra-molecular proton transfer (ESIPT) have emerged as a class of promising luminescent materials in many practical applications, such as optical memories¹ and switches,² UV absorbers,³ laser dyes,⁴ radiation detection scintillators,⁵ white light OLEDs⁶ and particularly fluorescent probes.⁷⁻¹⁸ ESIPT molecules can undergo a fast ($k \approx 10^{12} \text{ s}^{-1}$) photo-tautomerization reaction to yield an excited keto form (K*), mediated by an intra-molecular hydrogen bonds (H-bonds) upon photo-irradiation¹⁹ (Scheme S1†), which are famous for an abnormal emission with large Stokes shift emission (100–200 nm) without self-absorption.

Among the reports on ESIPT molecules in probe design, derivatives of 2-(2-hydroxyphenyl)benzoxazole (**HBO**) and 2-(2-hydroxyphenyl)benzothiazole (**HBT**) are the most attractive showing remarkable results with very high selectivity and sensitivity for detection of cellular transmembrane potential,^{7,8} apoptosis,⁹ $Mg^{2^+,10}$ protein tyrosine,¹¹ F^- ,^{12,18} cysteine and homocysteine,¹³ Zn^{2^+} (ref. 14, 16 and 17) and $Pd^{2^+,15}$ However, there are still some critical aspects that should be improved: (i) ESIPT emissions are usually sensitive to protic/polar solvents^{11,20} and often inhibited with only normal emission (at

much shorter wavelengths); and (ii) very low fluorescence quantum yields of 0.02 (for HBO) and 0.005 (for HBT).¹⁹ All these problems have severely limited their applications in biosensing and bio-imaging.²¹ Many efforts have been devoted to increase fluorescence quantum yields of these ESIPT molecules by introducing bulky dendrimer structures to inhibit the isomerization efficiency;22 or by introducing moieties with a Hbond for variation of inter- and intramolecular interactions to restrict molecular motions.^{23,26} On the other hand, aggregation induced enhanced emission (AIEE) fluorophores have shown distinct advantages in many areas of applications such as chemo/biosensors²⁴ and OLEDs.²⁵ ESIPT materials with AIEE properties have also been developed^{23,26} due to high quantum yields in aggregation states and avoidance of interactions with solvents. It is very important to generate such homo-dispersed aggregation of nanoparticles in solution for detection applications. Kim et al. obtained organic nanoparticles in a poor solvent with almost exclusively the abnormal emission assigned to the keto tautomer.11 However, the high critical concentration of the ESIPT molecules for the keto-emission results in a turbid solution, which is not favourable for the detection efficiency. Yang, et al. solved the above problem by dispersing the probes in the CTAB micelle with smaller aggregates and consequently clear solution, in which high reactivity towards F⁻ was observed.12 The probing medium was further extended to pure water by dispersing an insoluble organic dye in PVP hydrogel substrates with enhanced sensing performance.18 Although the advances are significant, the preparation of the detection systems is complicated and time-consuming. Thus, it is still of significance to develop new ESIPT dyes which can form homodispersed and stable nanoparticles in aqueous solution with more simplicity and higher quantum yields.



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[†] Electronic supplementary information (ESI) available: Schematic representation of the ESIPT photocycle, synthetic routes of the **HBI**s and **HBO**s, and crystal data for **DPIN**. CCDC 983882. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4tb00190g

It is well known that HBIs (2-(2-hydroxyphenyl)benzimidazole derivatives) have much higher quantum yields than HBOs and HBTs.19 Nevertheless, to the best of our knowledge, very few derivatives or analogues of HBI have been photophysically studied and rare fluorescent probes based on them have been reported by taking advantage of the ESIPT originated emission.²⁹ There are two drawbacks that hampered the development of HBIs displaying ESIPT emissions in probe design: (i) the keto emission of classical HBIs locates mainly in the blue region (470 nm), which exhibited much smaller Stokes shift compared with HBOs (500 nm) and HBTs (520 nm); (ii) the potential interference from other cations or anions towards the binding-able site (NH, N=) of the imidazole ring would block intramolecular H-bonds involved in the ESIPT process, which severely reduces quantum yields of the ESIPT emission. In this work, we tried to modify the structure of HBIs to improve the ESIPT emission performance by not only increasing their Stokes shifts but also quantum yields. With a simple one-pot reaction, we obtained DPIN (3-(4,5-diphenyl-1H-imidazol-2-yl)naphthalen-2-ol, Scheme 1). The compound exhibited obvious ketoform emission even in some polar and protic solvents (DMF, DCM and ethanol) at low concentration with large Stokes shift and a high quantum yield up to 0.30 in DMF. Transparent solution can be obtained by dispersing DPIN in water in which stable homo-dispersed aggregated nanoparticles of DPIN were formed, showing almost exclusive keto-emission with a quantum yield of 0.20. The fluorescence stability of DPIN in water was also demonstrated by interference experiments involving factors such as pH value and ions (cations and anions).

An elevated level of biothiols (Cys, GSH and HCy) could bring out different diseases, however, they are difficult to be distinguished because of their similarity in structures.²⁸ For sensors displaying the ESIPT, the signaling transduction is often achieved by the turn-on fluorescence of keto-form emission upon



Scheme 1 Synthetic routes of DPIN and the target probe DPIN-A and the reaction process of the probe towards Cys (M: $NH_4Ac/HAc/reflux$).

response to the analytes.¹¹⁻¹⁸ Based on the excellent fluorescence performance of **DPIN**, we designed a new probe denoted as **DPIN-A** (Scheme 1) by protecting the OH-moiety of **DPIN** with an acryloyl group which can react with biothiol Cys selectively. The results show that **DPIN-A** has a high selectivity and sensitivity towards Cys in pure water which is of important significance in clinical diagnose.

2. Results and discussion

2.1 Design of HBIs and organic probe for Cys

DPIN was designed based on **HBIs** with following considerations: (i) **HBIs** have a high fluorescent quantum yield of about one order of magnitude higher than that of **HBOs** and **HBTs**; (ii) the NH of the imidazole core as a H-bonding donor would enhance the intermolecular interaction which is beneficial for molecular aggregations; (iii) the two phenyls as tails of imidazole with a large twisted angle would restrict intermolecular π - π stacking thus inhibiting aggregation caused fluorescent quenching; (iv) expanding the π -conjugation length by introducing the 3-OH-2-naphthalene ring as the head of the molecule would result in a keto emission in longer wavelength with larger Stokes shift, with which the distinct colour difference compared with normal emission would be discerned by naked eyes.

By protecting the OH of **DPIN** with an allyl group, we synthesized the probe **DPIN-A** (Scheme 1) with enol emission quenched due to the PET (Photoinduced Electron Transfer) process between the skeleton and the propylene moiety. After the conjugate addition of the three similar biothiols with acrylates, it will generate thioethers which will interrupt the PET process with enol-like emission. It can further undergo an intramolecular cyclization to free the OH of **DPIN-A** which can undergo the ESIPT process with keto emission (Scheme 1). **DPIN-A** was expected to have a high selectivity towards Cys over HCy and GSH for the different intra-molecular cyclization rates.¹³

2.2 Synthesis and crystal structures

Synthetic routes of DPIN and the target probe are outlined in Scheme 1. Another four ESIPT dyes of HPO-1, HPO-2, HPI-1 and HPI-2 were synthesized for comparison (Scheme S2[†]). DPIN, HPO-1, and HPO-2 were obtained by refluxing benzyl/phenanthrene-9,10-dione, araldehyde, NH₄Ac in HAc as the solvent. HPI-1 and HPI-2 were obtained by refluxing phenanthrene-9,10-dione and araldehyde together in the mixed solvent of CH₂Cl₂-ethanol, catalyzed by a small quantity of HAc in one pot reaction (for details, see Experimental section). The resulting compounds were characterized by ¹H-NMR, elemental analysis, and MS. Single crystals of DPIN were obtained by layering hexane on CH₂Cl₂. X-ray single-crystal diffraction analysis revealed that DPIN crystallized in a triclinic, p-1 group. As shown in Fig. 1, three different kinds of weak interactions were observed: (i) the short distance of 1.7 Å between H and N indicated strong intramolecular O-H···N hydrogen bonds, which favours the mobility of the proton from -OH to Lewis



Fig. 1 Crystal packing structure of DPIN, dot lines represent weak intermolecular interactions: O-H…N (green), N-H…O (red), C-H… π (blue).

base ==N sites in the imidazole centre. (ii) The intermolecular N-H···O hydrogen bond with a distance of 2.0 Å and (iii) intermolecular C-H··· π interactions within a distance of 3.08–3.29 Å are beneficial for the molecular aggregation in the solution with a high concentration and an insoluble solvent. As expected, the intermolecular π - π stacking was suppressed due to the large twisted dihedral angle between the phenyl and imidazole rings (*ca.* 51.87° or 43.97°). The crystal stacking is shown in Fig. S1.† Selected dihydral angles and weak interactions are given in Table S1.† Structural parameters and data are listed in Tables S2 and S3.†

2.3 Emission of HBIs

Strong keto emission with large Stokes shift in polar/protic solvents are important for ESIPT molecules. As shown in Fig. 2, DPIN exhibited strong keto emissions (maximized at >540 nm) with large Stokes shifts in normal strong polar and protic solvents, except that in methanol. Different colours were observed from blue, green, yellow and red under the radiation under a UV lamp with 365 nm, due to different ratios between keto and enol emissions in different solutions. The bathochromic-shift of the keto-form emission in different solvents (553, 610 nm in DMF and cyclohexane, respectively) is well in accordance with the $n-\pi^*$ transition character of the keto tautomer. However, at a low concentration of 10 μ M, the enol emission at ~415 nm is very weak, while the keto emission is strong with large Stokes shift peaked at \sim 553 nm even in polar (DMF) and protic solvents (ethanol) with yellow and green emissions, respectively. In nonpolar solvents the emissions show orange yellow at \sim 600 nm for benzene and DCM and red (~610 nm) for cyclohexane. To elucidate the origination of the excellent fluorescence performance of DPIN, emission spectra of four other molecules with similar structures (HPO-1, HPO-2, HPI-1 and HPI-2) were also characterized and are shown in Fig. S2.† The emissions of HPO-1 and HPO-2 showed much larger dependence than HPI-1, HPI-2 and DPIN on solvents with just moderate keto-form emission in non-polar solvents. After careful examination of their structures, we deduce that the less dependence on solvents of the keto-emission of DPIN and HPI-1 and HPI-2 might be mainly attributed to the molecular aggregation due to the presence of -NH within the imidazole ring





Fig. 2 Fluorescence of DPIN (10 μ M) in different solvents excited at 371 nm (top); pictures of DPIN in different solvents (10 μ M) excited with a hand lamp at 365 nm (down).

which can act as a hydrogen donor for intermolecular H-bonding interactions. HPO-1, HPO-2, HPI-1 and HPI-2 showed smaller Stokes shift than DPIN in spite of the involvement of more conjugated 9,10-phenanthrenequinone instead of benzyl as the tail of these molecules. It has been demonstrated that the highest occupied molecular orbitals (HOMO) of the keto form of HPI are mainly located at the imidazole ring and the head substituents which extend their conjugation length can reduce the energy gap efficiently.6 The introduction of 3-OH-2-naphthalene as the head of DPIN is essential to the large Stokes shift of the keto-emission for its longer conjugation length than 2-OH-naphtanlene and benzene. These results prove that the -NH in the imidazole ring and 3-OH-2-naphthalene are crucial for the stable keto emission with large Stokes shift. Compared with other ESIPT molecules, the excitation and emission of **DPIN** at longer wavelength (ex, \sim 371 nm; enol emission, ~415 nm; keto emission, ~550 nm) are much more beneficial for biosensing and bioimiaging.11-18

2.4 Emission performance of DPIN in DMF with different concentrations

Many organic dyes suffer aggregation caused quenching (ACQ) in concentrated solution or solid states for the π - π stacking. To avoid ACQ, we introduced benzyl with large twisted angles into **DPIN**. As shown in Fig. 3, the fluorescent spectra at a low concentration of 2.5 μ M in DMF mainly at ~480 nm which originated from the species resulting from the interaction of the solvent with **DPIN**. Two shoulders at 415 nm and 565 nm can be



Fig. 3 PL of **DPIN** in DMF with different concentrations ranging from 2.5 μ M to 100 μ M under 371 nm excitation. Inset picture: emission colour of **DPIN** in DMF with different concentrations, excited under a 365 nm hand lamp.

assigned to enol emission and keto emission, respectively, according to previous reports. Upon increasing the concentration from 2.5 μ M to 100 μ M, the intensity of keto emission mainly at 565 nm appears and increases rapidly. This was further demonstrated by the colour change discerned by naked eyes from blue (2.5 μ M), yellow (10 μ M) to bright yellow (50 μ M) under 365 nm radiation (Fig. 3, inset). This enhanced keto tautomer emission can be attributed to the increased planarity and rigidity between imidazole and naphthalene rings as well as the restriction of the molecular motion by the J-aggregation with a head-to-tail arrangement which is more facile for the tautomerization of **DPIN** from the enol-form to the keto-form upon photo excitation.^{18,23}

2.5 Effect of H₂O fraction on emission performance

We also investigated addition of H₂O on the fluorescence performance of **DPIN** in DMF solution (10 µM). The fluorescence spectra showed an obvious change upon increasing the ratio of H₂O from 0 to 100% (Fig. 4a). A similar fluorescent performance of **DPIN** in THF with different H₂O proportions is shown in Fig. S3.† In pure DMF, besides the strong keto-form emission at ca. 565 nm and weak enol-form emission at ca. 415 nm, the emission spectra of DPIN displayed a third weak peak at ca. 480 nm which might be due to the formation of species that are intermolecularly hydrogen-bonded to DMF molecules. Upon addition of water, this peak disappeared and the intensity of enol-form emission increases upon the concentration of H₂O up to 70% while the keto-form emission showed an irregular change. The decreasing intensity ratio between the keto- and enol-form emissions below addition of water up to 80% may be due to the formation of the hydrogen bond between DPIN and water, which is an obstacle for the tautomerization of DPIN from the enol form to the keto form. The keto-form emission becomes more prominent up to the concentration of 90%,



Fig. 4 (a) Fluorescence of **DPIN** in DMF and H₂O mixed solution (10 μ M), with a proportion of H₂O increasing from 0 to 100% water v/v in DMF. Excited wavelength: 371 nm. Inset picture: fluorescence of **DPIN** solution with different contents of H₂O, excited under a 365 nm hand lamp. (b) Ratio of intensity of keto emission toward enol emission. (c) Size distribution of **DPIN** in pure water by DLS.

which may be the critical concentration for the aggregation of **DPIN** to nanoparticles (Fig. 4b). Only keto-form emission can be observed in 100% water, which can be attributed to the formation of nanoparticles. Consequently, the emission colour can be varied from yellowish green (0%), yellow (60%) to green (100%) with different water contents, which are in well accordance with the spectral change (Fig. 4a, inset). DLS analysis (dynamic light scattering) (Fig. 4c) showed that the size of the nanoparticles is about 205 nm in pure water. The formed solution is stable which is still clear after standing for 12 h with a negligible change in the emission spectra (Fig. S4†).

The absolute quantum yields of keto-emission of **DPIN** in DMF, pure water and DMF–H₂O mixed solution (10 μ M, v/v, 2:3) were measured by using an integrating sphere. It shows high quantum yields of 0.30 (keto emission quantum yield: 0.28), 0.45 (keto emission quantum yield: 0.39), and 0.20 in DMF, DMF–H₂O (2:3) and pure water, respectively. These are much higher than that of the reported AIEE ESIPT molecules.²³

2.6 pH effects

As known, **HBI**s have amphoteric properties due to the presence of both Lewis acidic -NH- and basic =N- sites in the imidazole ring. It is very important to examine the pH effect on the fluorescence performance of **DPIN**. The experiments were conducted in PBS buffer (20 mM). The results showed that the performance of the fluorescence of **DPIN** hardly changed under different pH values from 6.0 to 8.8 (Fig. 5a). The experiment results indicate that **DPIN** is a good candidate dye displaying ESIPT for applications in the physiology environment. Paper

Fig. 5 The fluorescent spectra of DPIN (10 μ M) in PBS buffer (20 mM): (a) with a pH from 6.0 to 8.8; (b) with common cations and ions (100 eq.) added, (pH, 7.2).

2.7 Interference of common ions

Imidazole behaves as not only an excellent hydrogen bond donor to an anion receptor but also an electron donor that can bind metal ions by coordination bonds. In other words, the fluorescence of dyes involving imidazole rings may be affected profoundly by the presence of ions.²⁷ On this account, it is necessary to investigate the effect of ions on the fluorescence performance of **DPIN**. The experiments were carried out in PBS buffer (20 mM, PH, 7.2) with common cations and anions. As shown in Fig. 5b, all the ions examined showed a negligible effect on the intensity of the emissions. It is concluded that the fluorescence performance of **DPIN** would not be disturbed by common ions.

2.8 Optical responses of probe DPIN-A towards Cys

In view of the excellent fluorescence performance of DPIN, we designed a new probe DPIN-A for Cys by protecting the -OH group with an allyl group. The formed acrylate quenched both the enol- and keto-form emissions due to the PET process and block the -OH group. Condensation of acrylates with Cys which could release the -OH group in two step reactions restored the DPIN emission. This fluorescent turn-on process had been reported to detect Cys in the CTAB micelle with an obvious color change discerned by eyes.12 Here, we investigated the sensing performance for Cys of DPIN-A in a more simple protocol. The experiments were carried out using a 10 µM solution of DPIN-A in pure water (PBS buffer, 20 mM, pH, 7.2). Fig. 6a shows the fluorescent spectra of the assay system in the presence of Cys (10 eq.) for 150 min. After the addition of Cys, the intensity of keto-form emission increases gradually, while that of enol-form emission increased quickly at first ($\sim 10 \text{ min}$) then decreased gradually. The emission intensity is relatively stable in about 20 minutes for both enol- and keto-emission (Fig. 6b). The enol emission increases initially due to the conjugate addition, which removes the alkene-induced PET quenching and the increase of the keto emission is due to the formation of lactam releasing DPIN exhibiting the ESIPT process.

We also investigated the fluorescent response of **DPIN-A** towards different amounts of Cys (0–100 μ M) after reaction for 30 min. The results (Fig. 6c) showed that the increasing intensity of the major peak at *ca.* 553 nm correlates with the increasing concentration of Cys. The intensity *vs.* concentration



Fig. 6 (a) The fluorescent spectra of DPIN-A (10 μ M) in PBS buffer (20 mM, PH, 7.2) in the presence of 10 eq. Cys from 0 to 150 min. (b) The fluorescent emission intensity for keto emission ($\lambda = 553$ nm) and enol emission ($\lambda = 425$ nm) as time increases. (c) The fluorescent spectra of keto emission in the presence of different amounts of Cys after 30 min. (d) The fluorescent intensity at 553 nm toward different amounts of Cys from 0–30 μ M.

of the Cys plot shows a linear correlation to the amounts of Cys from 0 to 30 μ M (Fig. 6d). The detection limit of Cys was calculated to be 0.21 μ M which is below the requisite detection limits for Cys assays,³⁰ indicating that **DPIN-A** is suitable for sensing Cys with good sensibility in pure water.

2.9 Selective detection of biothiols

To evaluate the selectivity of DPIN-A towards Cys, we studied the fluorescence performance of DPIN-A corresponding to biothiols (10 eq.) and other amino acids (100 eq.) in pure water (PBS buffer, 20 mM, pH, 7.2). The emission spectra changed slightly compared with the blank sample in the presence of other 19 kinds of amino acids, indicating that the other amino acids did not react with DPIN-A. Biothiols including HCy, Cys and GSH have similar structures which just differ by a single methylene unit in their side chains.²⁸ It can be seen that (Fig. 6b and 7a) all of them can give rise to enol emission ($\lambda = 425$ nm) indicating their conjugate addition reactions with DPIN-A. Interestingly, only the addition of Cys can significantly increase the intensity ratio between the keto- and enol-form emissions. It displays strong emission at \sim 553 nm while the enol emission peaked at \sim 425 nm is very weak. The emission intensity change of **DPIN-A** towards HCy and GSH with time are shown in Fig. S5.† Compared with Cys, the emission at 425 nm for HCy and GSH was much stronger than that of Cys, indicating that they cannot be removed quickly after conjugation with DPIN-A. The high selectivity of Cys over HCy and GSH was further proved by the ratio between emission intensity at 553 nm and 425 nm as time increases (Fig. 7b). For Cys, it reached the stable ratio about 12 after 20 min with yellowish-green emission, whereas HCy and GSH show a much lower ratio (below 2.0) with blue emission



Fig. 7 (a) Fluorescent spectra of DPIN-A (10 μ M) towards Cys (10 eq.), HCy, (10 eq.) GHS (10 eq.). The spectra were recorded after 30 min. Other amino acids (100 eq. obtained after 1 hour.) (b) The ratio of fluorescent intensity centred at 553 nm and 425 nm towards Cys, HCy and GSH with a function of time. (c) Intensity ratio (of keto emission and enol emission) of the probe (10 μ M) towards other amino acids (100 eq., data were obtained after 1 hour) and biothiols of HCy, GSH and Cys (10 eq., data were obtained after 30 min).

(Fig. 7b inset). In Fig. 7c we can directly see that the emission intensity ratio of keto *vs.* enol is high up to \sim 14 towards Cys of **DPIN-A** after reaction for 30 min. It has a higher selectivity over other amino acids and biothiols, which exhibits a ratio below \sim 2.0 even after a longer reaction time of 1 h.

2.10 Interference experiment

To further investigate the selectivity of Cys over other competitive species, interference experiments of other amino acids towards Cys were carried out. The intensity ratio between 553 nm and 425 nm of the probe (Fig. 8) displays no distinct



Fig. 8 Bars represent the final fluorescence intensity at 528 nm in PBS buffer (20 mM, pH, 7.2). Red bars: emission responses of DPIN-A towards amino acids (100 eq.). Green bars: emission responses of DPIN-A (10 μ M) towards amino acids (100 eq.) in the presence of Cys (10 eq.) All the data were obtained after 1 h.

change after being cultured with excessive other amino acids (100 eq.) for about one hour (red bars). Upon addition of 10 eq. Cys in the buffer solution of **DPIN-A** together with other amino acids (100 eq.) for about an hour, the intensity ratio increased significantly (green bars), indicating that **DPIN-A** can selectively detect Cys without interference from other amino acids.

3. Experimental section

3.1 Materials and general methods

¹H-NMR spectra were recorded using a Varian Gemin-400 MHz spectrometer. Fluorescence spectra and absolute quantum vields (measured with an integrating sphere) were measured using an optical spectrometer (PTI: QM-TM, USA). MS analyses were recorded using a Perkin Elmer Flexar SQ 3000 mass spectrometer. Elemental analysis (C, H and N) was performed using a Vario EL III CHNS elemental analyzer. The single crystal structure of DPIN was obtained by X-ray diffraction with a Gemini A Ultra X-ray single crystal diffractometer using graphite monochromatized Cu-Ka radiation (Cu-Ka, $\lambda = 1.54184$ Å). All pH measurements were made with a Sartorius (PB-10) pH meter. Dynamic Light Scattering (DLS) measurement was performed using a nano Zetasizer (Malvern). Flash chromatography was performed with silica gel (200-300 mesh). Spectroscopic grade DMF and Milli-Q water (18.2 MΩ cm, 25 °C) were used throughout the detection experiments.

3.2 Synthesis of 3-hydroxy-2-naphthaldehyde

3-Hydroxy-2-naphthaldehyde was synthesized according to the previous reported literature with little modification.³¹ In a dry 100 mL round bottom flask was typically charged with 6 mmol of the naphthalen-2-ol, dissolved in 3 mL of anhydrous THP under N2 protection. Then 5.6 mmol of 1.6 M t-BuLi in pentane was added dropwise via a syringe in approximately 2 min to the stirred solution at room temperature. Immediately a rapid evolution of heat and gases occurred, thus raising the temperature of the reaction mixture to ca. 50 °C. After the gas evolution subsided (15 min), the reaction mixture was left to stir for another 4 h. A solution of the DMF (3 mL) in THP (3 mL) was then added to the above mixture at the 0 °C and, subsequently left to stir for 24 h at room temperature. After the reaction finished, water was added carefully to the mixture and dropped 1 M HCl until pH was 5-6. The mixture was extracted with ethyl acetate, dried over anhydrous Na2SO4 and evaporated to dryness. The resulting residue was chromatographed on silica gel (petroleum ether-CH₂Cl₂, 3 : 1), and a bright yellow powder was then obtained with 60% yield. $^1\mathrm{H}$ NMR (400 MHz, DMSO) δ 7.30 (s, 1H), 7.36 (ddd, 1H), 7.55 (ddd, 1H), 7.77 (d, 1H), 7.99 (d, 1H), 8.36 (s, 1H), 10.42 (s, 1H), 10.59 (s, 1H).

3.3 Synthesis of 3-(4,5-diphenyl-1*H*-imidazol-2-yl) naphthalen-2-ol (DPIN) (1)

The product was prepared by refluxing benzyl (0.21 g, 1 mmol), 3-hydroxy-2-naphthaldehyde (0.32 g, 1.2 mmol) and ammonium acetate (0.75 g, 10 mmol) in glacial acetic acid (50 mL) for 24 h under an argon atmosphere. After cooling to room temperature, a pale yellow mixture was obtained and poured into a methanol solution under stirring. The separated solid was filtered off, washed with methanol, and dried to give a light yellow solid. The solid was purified by column chromatography (petroleum ether-CH₂Cl₂, 1 : 1) on silica gel. ¹H NMR (400 MHz, DMSO-d₆, TMS) δ 5.32 (d, J = 4.7 Hz, 1H), 7.19–7.67 (m, 12H), 7.78 (dd, J = 18.1, 8.0 Hz, 2H), 8.66 (s, 1H), 12.93 (s, 1H), 13.32 (s, 1H). MS (ESI): m/z calculated 362.46, found 362.76. Anal. found: C, 82.36; H, 5.08; N, 7.45. Calc. for C₂₅H₁₈N₂O: C, 82.85; H, 5.01; N, 7.73%.

3.4 2-(Phenanthro[9,10-d]oxazol-2-yl)phenol (HPO-1)

HPO-1 was synthesized using a similar approach for **DPIN**. ¹H NMR (400 MHz, DMSO) δ , 7.17 (ddd, *J* = 15.1, 8.3, 4.3 Hz, 2H), 7.41–7.66 (m, 1H), 7.73–7.99 (m, 4H), 8.25 (dd, *J* = 7.8, 1.5 Hz, 1H), 8.44 (dd, *J* = 7.9, 1.1 Hz, 1H), 8.58 (dd, *J* = 7.8, 1.2 Hz, 1H), 9.01 (t, *J* = 8.6 Hz, 2H), 11.17 (s, 1H). MS (ESI): *m*/*z* calculated 311.33, found 311.46. Anal. found: C, 80.94; H, 4.43; N, 4.31. Calc. for C₂₁H₁₃NO₂: C, 81.01; H, 4.21; N, 4.50%.

3.5 2-(1*H*-Phenanthro[9,10-*d*]imidazol-2-yl)phenol (HPI-1)

9,10-Phenanthrenequinone (2.12 g, 10 mmol) and ammonium acetate (0.75 g, 10 mmol) were added to the 30 mL mixed solvent of ethanol and dichloromethane (1:1, v/v). After refluxing for 10 min, salicylaldehyde (0.32 g, 1.2 mmol) and a catalytic amount of glacial acetic acid were added. The reaction mixture was held at reflux for another 3 h. After cooling to room temperature, the mixture was filtered. The solid was purified by column chromatography (petroleum ether- CH_2Cl_2 , 1:1) on silica gel. A white powder was finally obtained after it was stirred in refluxing ethanol, subsequently filtered, and dried under vacuum. ¹H NMR (400 MHz, DMSO) δ 5.32 (s, 1H), 7.09 (ddd, J =4.5, 3.5, 1.8 Hz, 2H), 7.47-7.28 (m, 1H), 7.90-7.57 (m, 3H), 8.26 (dd, J = 8.1, 1.6 Hz, 1H), 8.56 (dd, J = 40.9, 7.7 Hz, 2H), 13.70 (s, 1H), 8.91 (dd, *J* = 12.7, 8.3 Hz, 2H), 13.14 (s, 1H). MS (ESI): *m*/*z* calculated 310.35, found 310.70. Anal. found: C, 81.16; H, 4.76; N, 8.94. Calc. for C₂₁H₁₄N₂O: C, 81.27; H, 4.55; N, 9.03%.

3.6 1-(Phenanthro[9,10-d]oxazol-2-yl)naphthalen-2-ol (HPO-2)

HPO-2 was synthesized using a similar approach for **DPIN**. ¹H NMR (400 MHz, DMSO) δ 7.42 (d, J = 9.0 Hz, 1H), 7.48 (t, J = 7.4 Hz, 1H), 7.63–7.75 (m, 1H), 7.77–7.93 (m, 3H), 8.00 (d, J = 7.4 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 8.44 (d, J = 7.7 Hz, 1H), 8.56 (dd, J = 11.4, 8.4 Hz, 2H), 9.04 (t, J = 8.6 Hz, 3H), 11.91 (s, 1H). MS (ESI): m/z calculated 361.39, found 361.75. Anal. found: C, 82.95; H, 4.65; N, 3.98. Calc. for C₂₅H₁₅NO₂: C, 83.09; H, 4.18; N, 3.88%.

3.7 1-(1*H*-Phenanthro[9,10-*d*]imidazol-2-yl)naphthalen-2-ol (HPI-2)

HPI-2 was synthesized using a similar approach for **HPI-1**. ¹H NMR (400 MHz, DMSO) δ 5.32 (d, J = 4.3 Hz, 1H), 7.26–7.44 (m, 7H), 7.46–7.52 (m, 1H), 7.52–7.62 (m, 4H), 7.88 (t, J = 8.5 Hz, 2H), 8.19 (d, J = 8.6 Hz, 1H). MS (ESI): m/z calculated 360.41, found 360.42. Anal. found: C, 83.60; H, 4.53; N, 7.85. Calc. for C₂₅H₁₆N₂O: C, 83.31; H, 4.47; N, 7.77%.

3.8 Synthesis of 3-(4,5-diphenyl-1*H*-imidazol-2-yl) naphthalen-2-yl acrylate (DPIN-A)

DPIN-A was obtained according to the reported literature.¹⁵ To a solution of **DPIN** (182 mg, 0.5 mmol) and Et₃N (2 eq.) in 10 mL of anhydrous CH_2Cl_2 , acryloyl chloride (1.25 eq., mixed with 4 mL of CH_2Cl_2) was added dropwise at 0 °C. After stirring at this temperature for 90 min, the mixture was warmed to room temperature and stirred overnight. The solvent was removed under vacuum to furnish a crude mixture which afforded **DPIN-A** upon flash chromatography as a light orange solid (110 mg, 51% yield) ¹H NMR (400 MHz, DMSO) δ 5.32 (s, 1H), 5.88 (s, 1H), 5.98–6.31 (m, 1H), 6.41–6.77 (m, 2H), 6.92–7.81 (m, 10H), 7.75–8.15 (m, 3H), 8.59 (s, 1H), 12.76 (s, 1H). MS (ESI): *m/z* calculated 416.47, found 416.81. Anal. found: C, 80.30; H, 4.90; N, 6.35. Calc. for $C_{28}H_{20}N_2O_2$: C, 80.75; H, 4.84; N, 6.73%.

4. Conclusion

In summary, by introducing benzyl as the tail, imidazole as the core and 3-OH-2-naphthalene as the head, we successfully designed and obtained an interesting ESIPT chromophore named **DPIN** which showed prominent ESIPT emission in protic and polar solvents with large Stokes shift and high quantum yields. The benzyl tail with a large twisted angle avoided aggregation induced quenching by restricting the intermolecular π - π stacking. The imidazole core supplies intermolecular H-bond sites which are in favour of J-aggregation in protic and polar solvents. 3-OH-2-naphthalene as the head of **DPIN** can extend the conjugation length effectively which results in a keto-emission with large Stokes shift.

DPIN can be dispersed in water forming stable transparent solution with almost exclusive keto-form emission and a high fluorescence quantum yield up to 0.20. The fluorescence performance is very stable in the physiology pH range of 6.2–9.2 and cannot be interfered by other common ions.

The new turn-on sensor denoted as **DPIN-A** (Scheme 1) by protecting the OH-moiety of **DPIN** with an acryloyl group showed high selectivity and sensitivity towards Cys and can discriminate Cys from other amino acids including GSH and HCy in pure water. Further studies are underway to optimize the sensing method.

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