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Supporting Information for

Unique phenanthrenequinone imidazole-based fluorescent materials with aggregation-induced or two-photon emission †

Yong Liu, Jie Niu, Weishan Wang, Baoli Dong and Weiying Lin *

Institute of Fluorescent Probes for Biological Imaging, School of Materials Science and
Engineering, School of Chemistry and Chemical Engineering, University of Jinan,
Shandong 250022, P.R. China.
E-mail: weiyinglin2013@163.com

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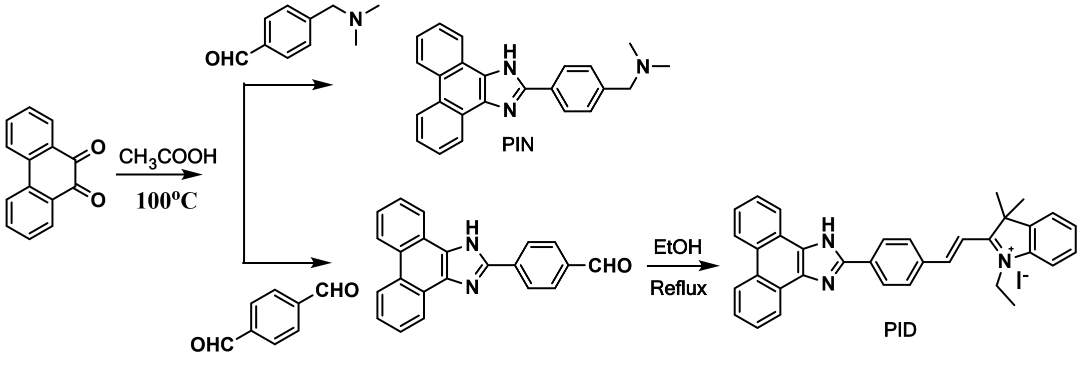
Materials

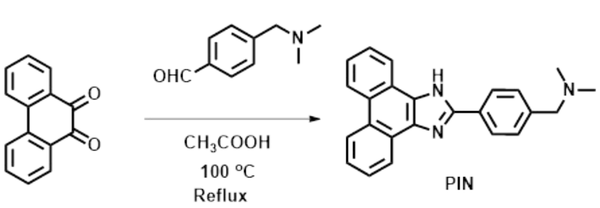
All reagents were obtained from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR spectrometer. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. Fluorescence imaging of the cells and tissues slices was obtained with Nikon A1MP two-photon confocal microscopy. Two-photon imaging was conducted on a SpectroPro300i and the pump laser beam came from a mode-locked Ti: sapphire laser system at the pulse duration of 200 fs, a repetition rate of 76 MHz (Coherent Mira900-D). All procedures for this study were approved by the Animal Ethical Experimentation Committee of Shandong Academy of Sciences according to the requirements of the National Act on the use of experimental animals (China). Cells were obtained from the College of Life Science, Nankai University (Tianjin, China).

Synthesis



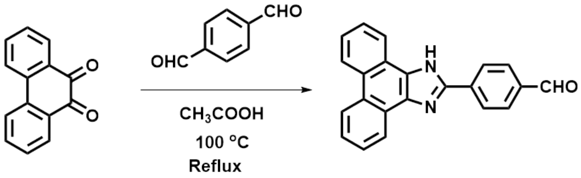
Scheme S1. The synthetic route to **PIN** and **PID.**

Synthesis of ***PIN***:



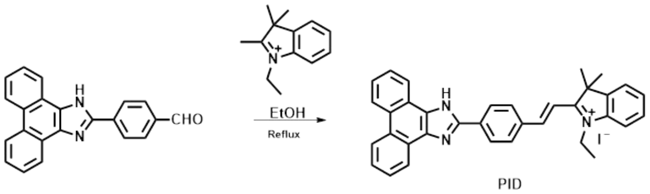
A mixture of 4-((dimethylamino)methyl)benzaldehyde (0.33g, 2 mmol), 9,10-phenanthroquinone (0.21 g, 1 mmol), and ammonium acetate (1.54g, 20 mmol) in glacial AcOH (20 mL) was heated to 100 ℃ for 5 h. The hot solution was cooled to room temperature, and the resulting yellow solid was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution. The white solid was further dried under reduced vacuum, and then purified by silica gel column chromatography using acetone as eluent to afford the pure product. ^{1}H NMR (400 MHz, MeOD) δ 8.80 (d, J = 8.2 Hz, 1H), 8.55 (s, 1H), 8.31 (d, J = 8.3 Hz, 1H), 7.75 – 7.61 (m, 3H), 4.12 (s, 1H), 2.72 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): 193.09, 148.17, 136.53, 135.91, 130.71, 127.77, 126.96, 29.50, 29.04, 27.01, 22.57, 14.43, 7.65. HRMS (m/z): $[M+2H]^+$calcd for $C_{24}H_{21}N_3$: 353.17; found, 353.16.

Synthesis of 4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde:



A mixture of terephthalaldehyde (0.27 g, 2 mmol), 9,10-phenanthroquinone (0.21 g, 1 mmol), and ammonium acetate (1.54g, 20 mmol) in glacial AcOH (20 mL) was heated to 100 ℃ for 5 h. The hot solution was cooled to room temperature, and the resulting yellow solid was collected by filtration and washed with acetate acid, dilute sodium hydrogen carbonate solution. The white solid was further dried under reduced vacuum, and then purified by silica gel column chromatography using acetone as eluent to afford the pure product. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 13.97 (s, 1H), 10.10 (s, 1H), 8.59 (s, 1H), 8.90-8.86 (t, J = 8.0 Hz, 2H), 8.71-8.69 (d, J = 8.0 Hz, 1H), 8.61–8.59 (d, J = 8.0 Hz, 3H), 8.14 – 8.12 (d, J = 8.0 Hz, 2H), 7.77 – 7.64 (m, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*): 193.06, 148.25, 136.47, 135.05, 130.57, 127.66, 127.14, 124.40.

Synthesis of ***PID****:*



Under the protection of nitrogen, the compound *4-(1H-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde* (0.19 g, 1.00 mmol) and 1-ethyl-2,3,3-trimethyl-3*H*-indol-1-ium (0.34 g, 1.1 mmol) were dissolved in 15 mL of EtOH and the resulting solution was allowed to react for 26 h at 80 °C. Ethanol in the mixture was evaporated, and then ether (30 mL) was poured into to form a red solid, which was washed by ether for several times, and the resulting solid was dried to afford the product **PID** as a red solid with a yield of 70 %. ^{1}H NMR (400 MHz, MeOD): δ 8.85 (s, 1H), 8.72 (s, 1H), 8.59 (s, 1H), 8.55 (s, 1H), 8.51-8.48 (d, J = 12.0 Hz, 2H), 8.34–8.32 (d, J = 8.0 Hz, 2H), 7.93 – 7.90 (m, 1H), 7.86 – 7.79 (m, 2H), 7.78 – 7.67 (m, 1H), 4.84– 4.78 (q, J = 8.0 Hz, 2H), 1.93 (s, 6H), 1.68 – 1.65 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*): 181.73, 153.37, 148.32, 144.58, 140.90, 138.08, 135.29, 134.65, 131.90, 130.04, 129.66, 128.60, 128.27, 127.74, 127.25, 126.86, 126.41, 126.07, 124.70, 124.35, 123.66, 122.84, 122.69, 122.47, 115.74, 113.17, 63.44, 52.86, 42.88, 30.24, 29.09, 23.46, 23.08, 14.43, 11.49. HRMS (m/z): [M-I+H]$^{+}$calcd for $C_{35}H_{30}N_3^{+}$: 493.2500; found, 493.2124.

*Synthesis of the control compound **PI***:

The compound **PI** was prepared according to reference literature.[1]

General procedure for the spectral measurement

For the compound **PIN**, the stock solution of the probe **PIN** was prepared at 1 mM in DMSO. The different pH (4.0-8.0) PBS solutions were prepared. The solutions of various testing species were prepared from Cys; Hcy; GSH; Al^{3+}; Fe^{3+}; Cu^{2+}; Zn^{2+} ; Ag^{+}; Cr^{2+}; Fe^{2+}; K^{+}; Ca^{2+}; Ni^{2+}; Na^{+}; Mg^{2+}; Co^{2+}; Pd^{2+}in the twice-distilled water. The test solution of the **PIN** (2 µM) in 5 mL PBS buffer (pH 7.4) was prepared. The titration and selectivity experiments, the excitation wavelength was 365 nm, and the excitation and emission slit widths were 5 and 5 nm, respectively.

For the compound **PIN,** the stock solution of the probe **PID** was prepared at 1 mM in DMSO. For the pH response experiments, the excitation wavelength was 405 nm, and the excitation and emission slit widths were 5 and 5 nm, respectively.

Quantum yields

The fluorescence quantum yields can be calculated by means of equation (1):

$$\Phi_s = \Phi_r \left(\frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left(\frac{{n_s}^2}{{n_r}^2} \right) \frac{F_s}{F_r} \qquad (1)$$

Where the subscripts *s* and *r* refer to the sample and the reference, respectively. Φ is quantum yield, F is the integrated emission intensity, A stands for the absorbance, and n is refractive index.

Cell viability evaluated by MTT assays

A549 cells were seeded per well in a 96-well plate. The next day the medium was changed into a medium containing **PID** and **PIN**. For the compound **PID and PIN**, incubated concentration (0 μM, 5 μM, 10 μM, 20 μM, 30 μM). And then the medium and the excess dyes were removed, and then 10 μL MTT (5 mg/mL in PBS) was added. After 24 h incubation at 37 °C, and 100 μL DMSO was added into the dishes to dissolve the formazan crystal product. The plate was shaken for 10 min, and then the absorbance at 490 nm was measured by the microplate reader. The cell viability (%) = $(\mathrm{OD_{490\ sample}} - \mathrm{OD_{490\ blank}})/(\mathrm{OD_{490\ control}} - \mathrm{OD_{490\ blank}}) \times 100\%$. $\mathrm{OD_{490\ sample}}$ denotes the cells incubated with the probe for different incubation time, $\mathrm{OD_{490\ control}}$ denotes the cells without the probe, $\mathrm{OD_{490\ blank}}$ denotes the wells containing only the culture medium.

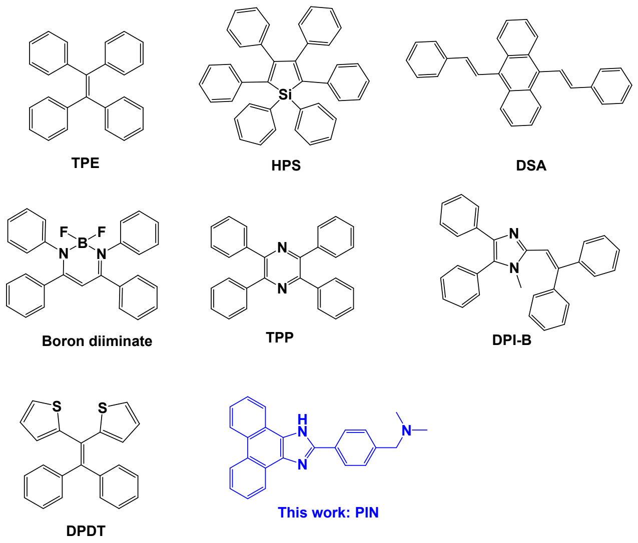


Fig. S1 Core structures of AIE dyes such as TPE, HPS, DSA, boron diiminates, TPP, DPI-B, and the newly developed **PIN**.

Table S1. Properties of the new **PIN** and the representative AIE materials previously reported.

Ref.	Core structure	Quantum yield in pure water	Electron-donating/withdrawing-Controlled	Emission wavelength	Application
This work	**Phenanthrenequinone imidazoles**	98 %	No	420 nm	Monitoring of Pd (II) by aggregation-disaggregation method
2	HPS	21%	No	496nm	Revealed the Aggregation-induced emission behaviours
3	HPS	56%	No	490nm	Revealed the phenomenon, mechanism and applications
4	HPS	56%	No	497nm	Exploration the development of OLEDs and sensory systems
5	HPS	39%	No	498 nm	Monitoring of pH value or DNA
6	HPS	_	No	497nm	Summarized AIE research
7	HPS	_	No	508 nm	Studied the competitive interaction between conjugation and rotation
8	HPS	_	No	490 nm	Monitoring of heparin with specific Proteins
9	HPS	_	No	479 nm	Monitoring of Cys, Hcy, GSH
10	HPS	_	No	480nm	Biosensor for integrin avb3
11	TPE	15.3%	No	477nm	Detection for protein
12	TPE	_	No	490nm	Studied a series of

					derivatives
13	TPE	14.5%	No	449nm	For nondoped OLED applications
14	TPE	_	No	530nm	A mechanistic study
15	TPE	44.2%	Yes	565nm	Demonstrated three TPE derivatives
16	TPE	_	No	490nm	Visualization of the dynamic interfacial evolution
17	TPE	_	No	539nm	Applications as field-effect transistors
18	TPE	_	No	500nm	Monitoring of H_2O_2
19	TPE	_	Yes	641nm	Two-photon excited imaging
20	TPE	_	No	500 nm	Monitoring of H_2S
21	TPP	_	No	466 nm	Three-Photon Microscopic Bioimaging
22	DSA	_	No	_	Report a new synthetic route
23	TPA	_	No	483 nm	Investigated the one- and two-photon optical properties of TPA derivatives in different solvents
24	TPA	_	Yes	560 nm	Application for OLED
25	BODIPY	_	Yes	530 nm	The pathways to a series of derivatives

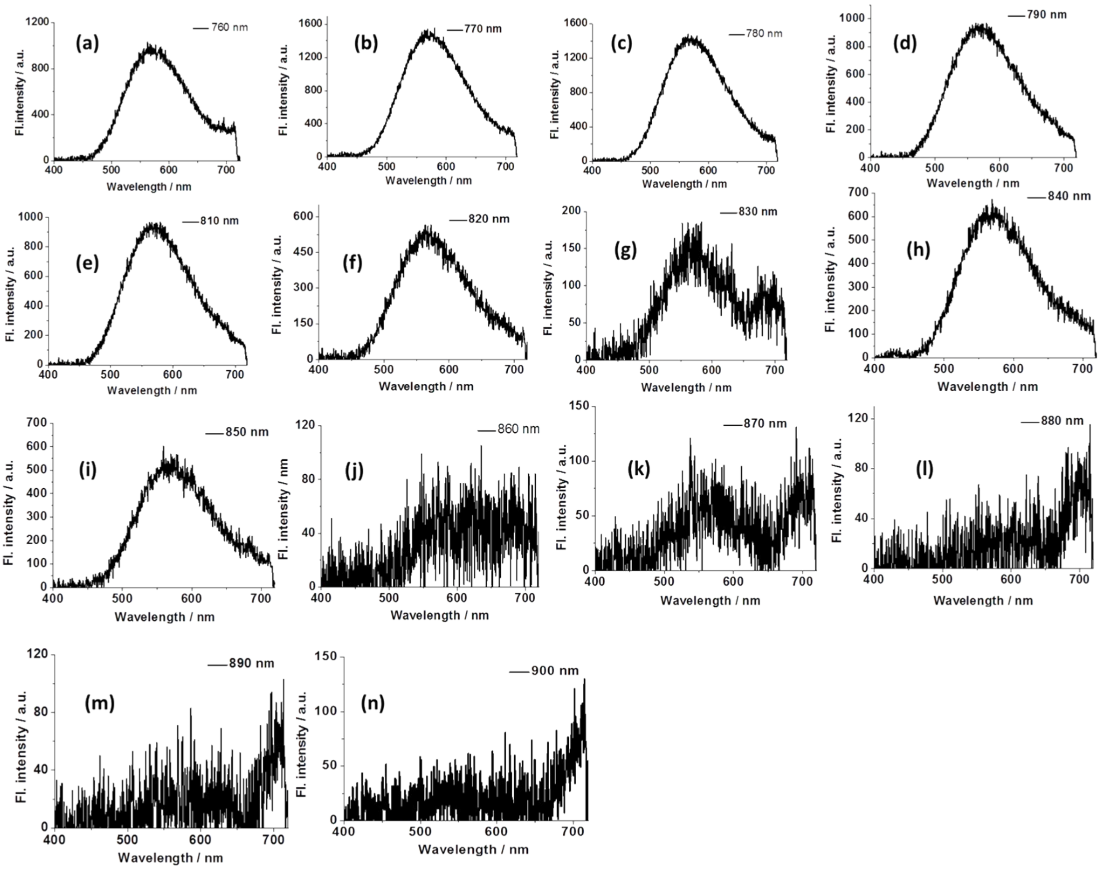


Fig. S2 TP fluorescence intensity of the compound **PID** (5 μM) at 760-900 nm.

Table S2. Photophysical properties of the compound **PIN**.

	$\Phi^a/\%$	δ^b/GM
PIN	98	-

^{a} refers to fluorescence quantum yield determined using fluorescein (Φ = 0.95) as the standard. ^{b} refers to two-photon absorption cross sections at 800 nm. 1GM = 10^{-50} cm^4 s photon^{-1}; [**PIN**] =10 μM. "-"refers to too small to be determined.

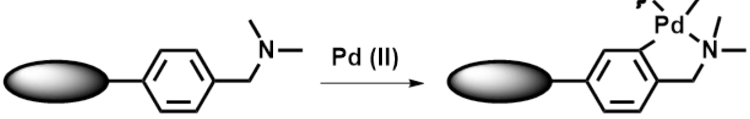


Fig. S3 Coordination of the aromatic ethylenediamine moiety to Pd(II).

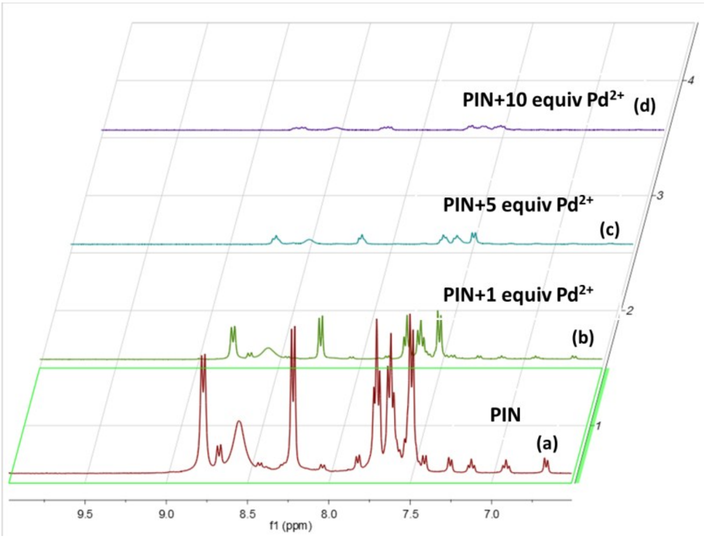


Fig. S4 (a) The ^{1}H NMR spectrum of **PIN** in d_6- DMSO/D_2O (4/1); (b) The ^{1}H NMR spectrum of addition of 1 equiv Pd (II) to **PIN** in d_6- DMSO/D_2O (4/1); (c) The ^{1}H NMR spectrum of addition of 5 equiv Pd (II) ions to **PIN** in d_6- DMSO/D_2O (4/1); (d) The ^{1}H NMR spectrum of addition of 10 equiv Pd (II) ions to **PIN** in d_6- DMSO/D_2O (4/1).

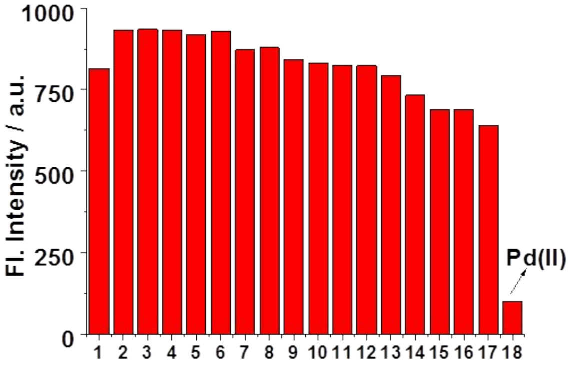


Fig. S5 Fluorescence responses of **PIN** (2 μM) in the presence of various relevant analytes. The concentrations of the representative analytes are 3 mM. Legend: 1. **PIN**; 2. Cys; 3. Hcy; 4. GSH; 5. Al^{3+}; 6. Fe^{3+}; 7. Cu^{2+}; 8. Zn^{2+}; 9. Ag^+; 10. Cr^{2+}; 11. Fe^{2+}; 12. K^+; 13. Ca^{2+}; 14. Ni^{2+}; 15. Na^+; 16. Mg^{2+}; 17. Co^{2+}; 18. Pd^{2+}. $\lambda_{ex} = 365$ nm.

Table S3. Cytotoxicity Data of **PIN** and **PID** at different concentration at 24 h. [a]

Incubated concentration (μM)	0	5	15	20	30
PIN:Survival	100%	91±4	90±4	86±4	81±4
PID:Survival	100%	91±4	91±4	87±4	80±4

[a] Cell viability was quantified by the MTT assays (mean ± SD).

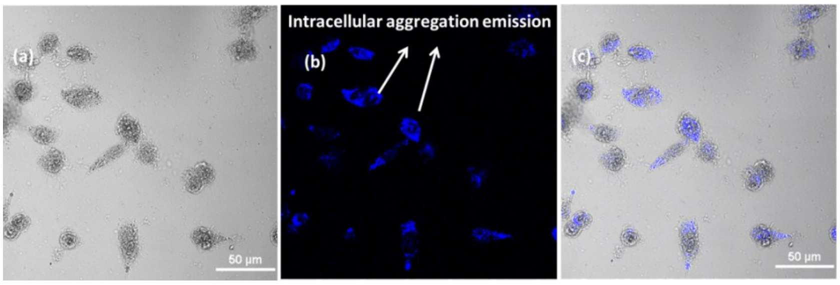


Fig. S6 (a) Bright-field image; (b) Fluorescence image of **PIN** (5 μM) collected between 425 and 475 nm upon excitation at 405 nm; (c) Merged image of a and c.

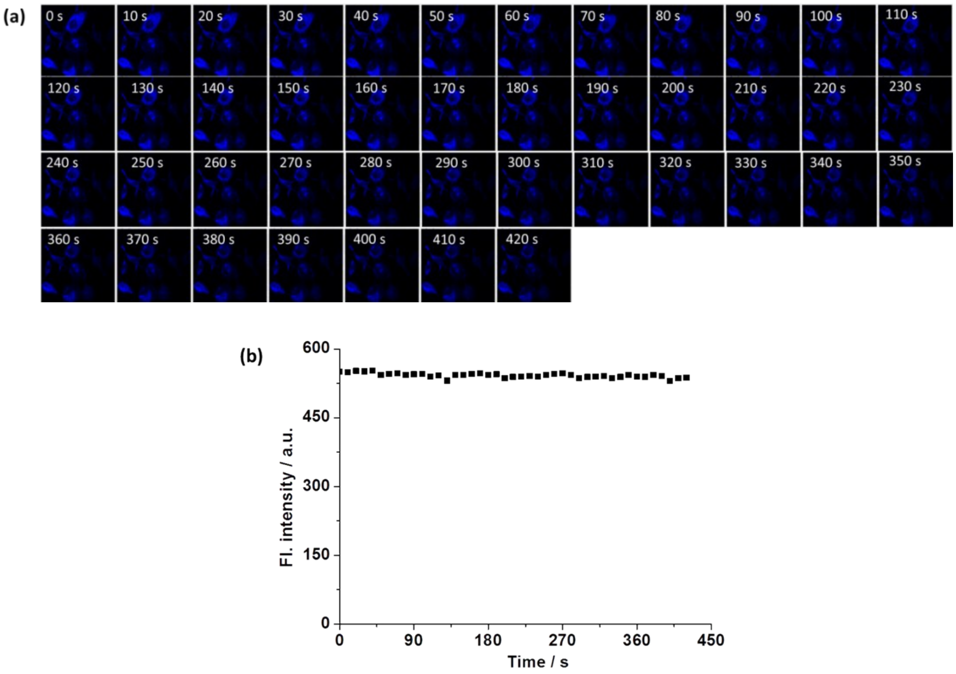


Fig. S7 (a) Fluorescence images of A549 cells incubated with **PIN** (5 μM) acquired at different times under successive excitation. (b) Mean intensities of the cells incubated with the probe in the green channel under successive excitation at different times. λ_{ex}: 405 nm, λ_{em}: 425-475 nm.

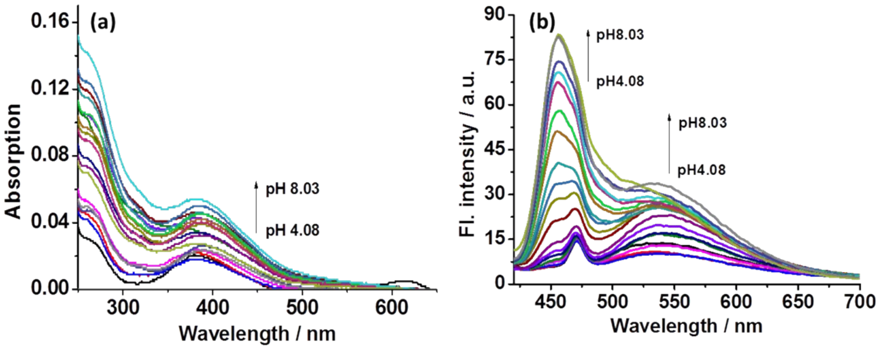


Fig. S8 (a) Absorbance and fluorescence (b) spectra of **PI-DI** (10 μM) in PBS buffer solutions with different pH values (4.0~8.0).

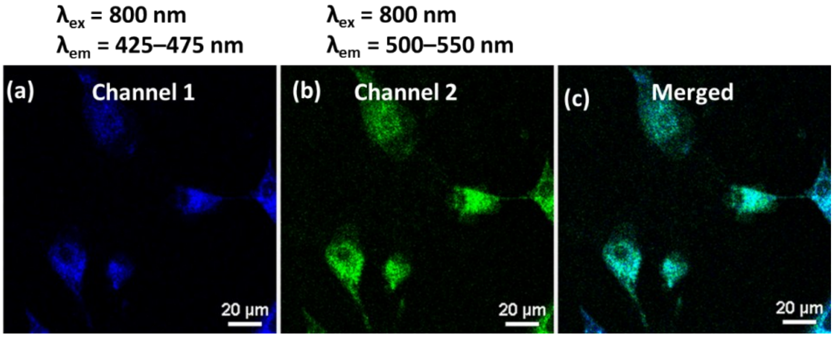


Fig. S9 Confocal TP fluorescence images of the living cells treated with **PID** (5 μM) for 30 min. (a) The blue emission channel (λ_{ex} = 800 nm, λ_{em}= 425-475 nm); (b) The green emission channel (λ_{ex} = 800 nm, λ_{em}= 500-550 nm); (c) Merged image of a and b. Scale bar = 20 μm.

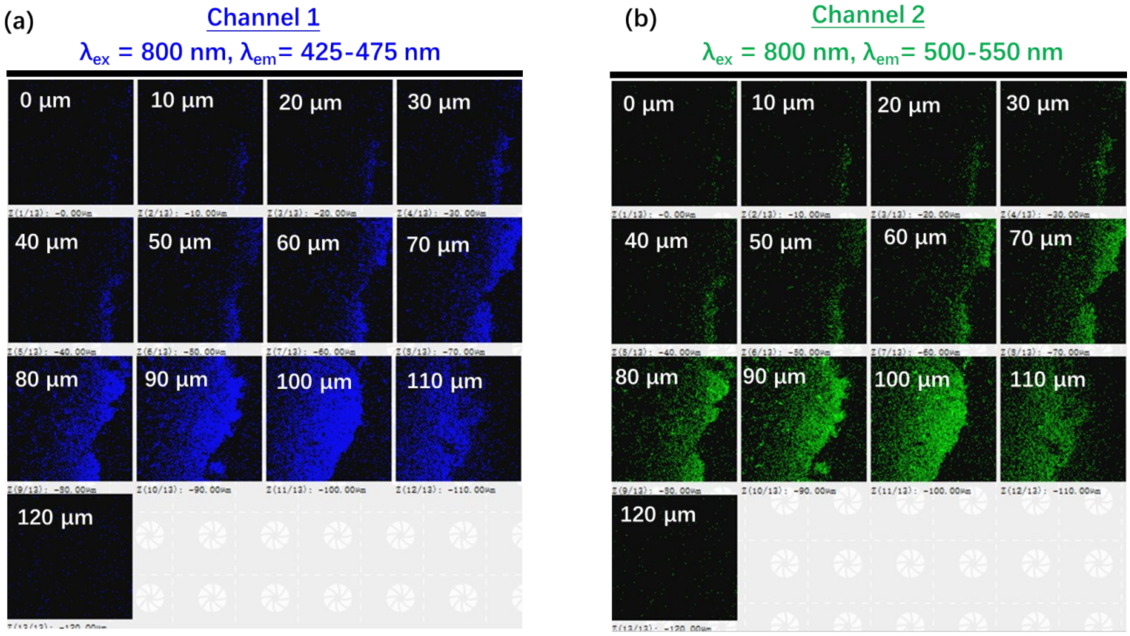


Fig. S10 Two-photon fluorescence images of mitochondrial pH in the mouse liver tissues incubated with **PID** (10 μM) for 1 h. (a) The blue emission channel (λ_{ex} = 800 nm, λ_{em}= 425-475 nm); (b) The green emission channel (λ_{ex} = 800 nm, λ_{em}= 500-550 nm).

Spectral characterization

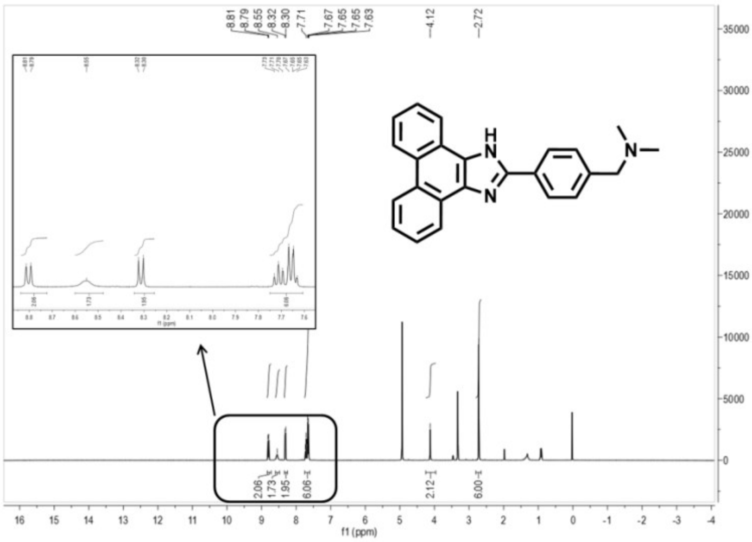


Fig. S11 ^{1}H NMR spectrum of the compound **PIN**.

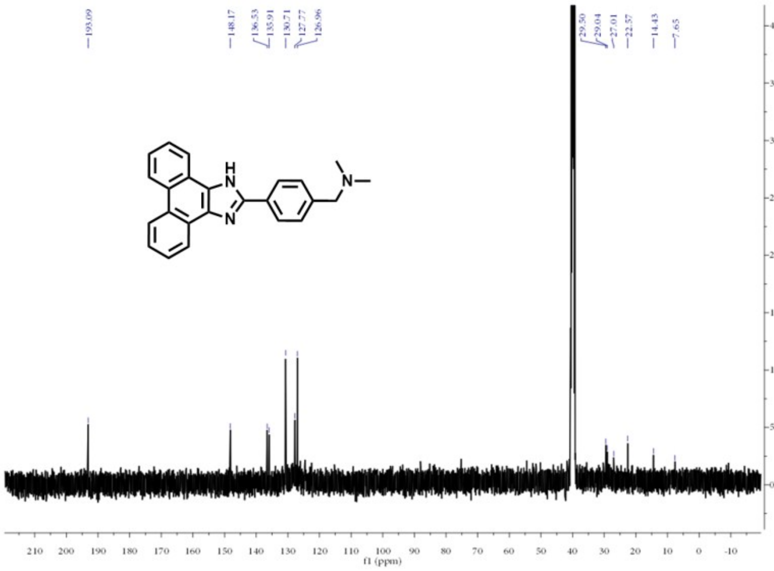


Fig. S12 ^{13}C NMR spectrum of the compound **PIN**.

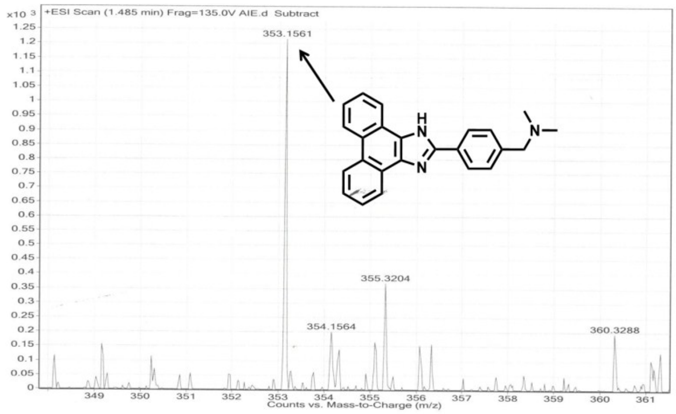


Fig. S13 HRMS spectrum of the compound **PIN**.

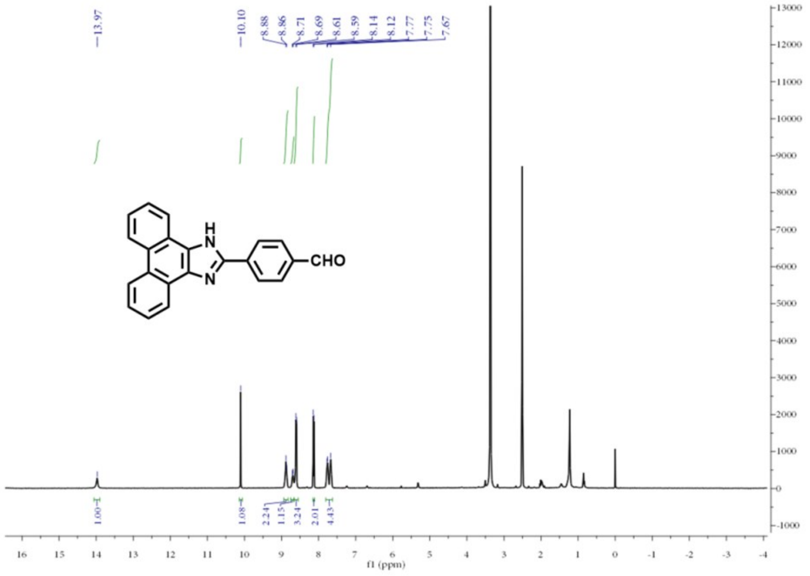


Fig. S14 ^{1}H NMR spectrum of the compound 4-(1*H*-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde.

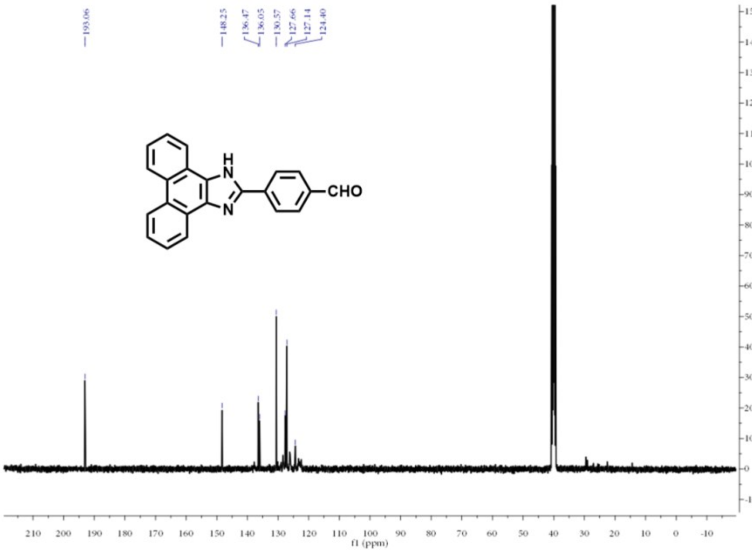


Fig. S15 ^{13}C NMR spectrum of the compound 4-(1*H*-phenanthro[9,10-d]imidazol-2-yl)benzaldehyde.

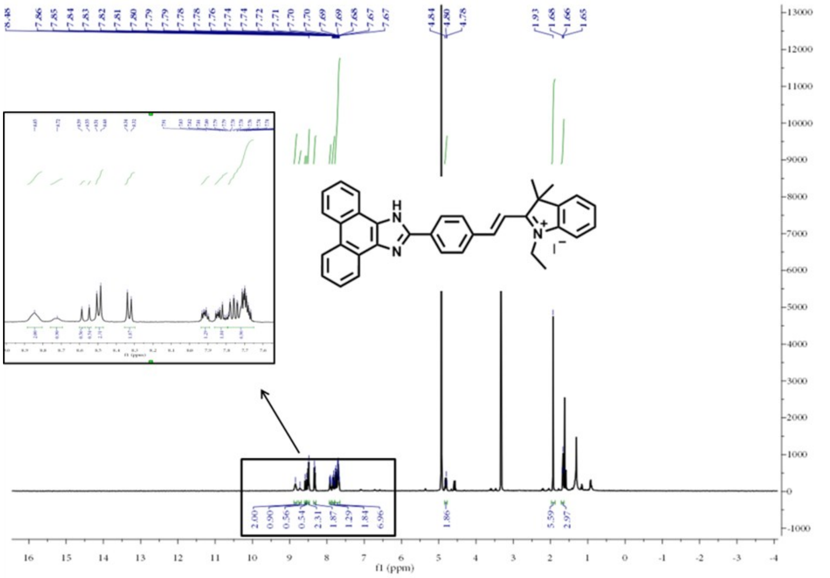


Fig. S16 ^{1}H NMR spectrum of the compound **PID**.

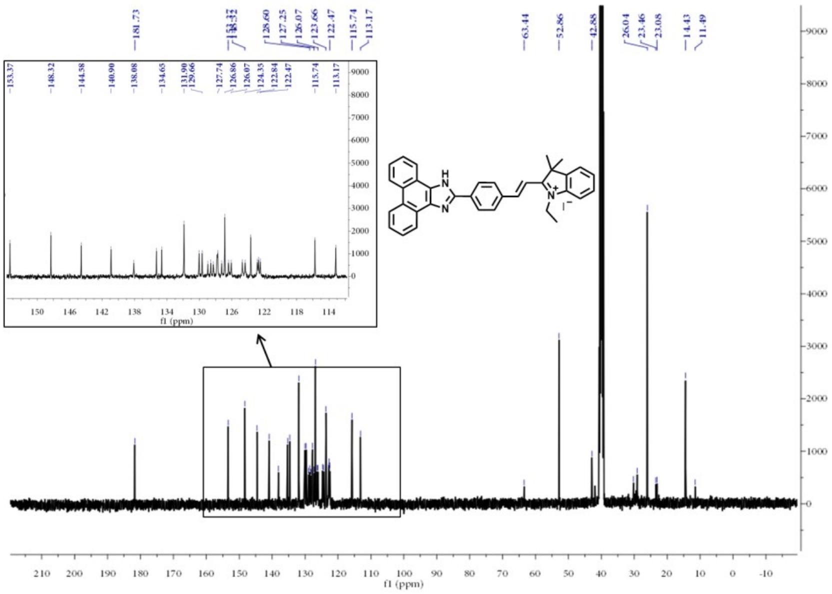


Fig. S17 ^{13}C NMR spectrum of the compound **PID**.

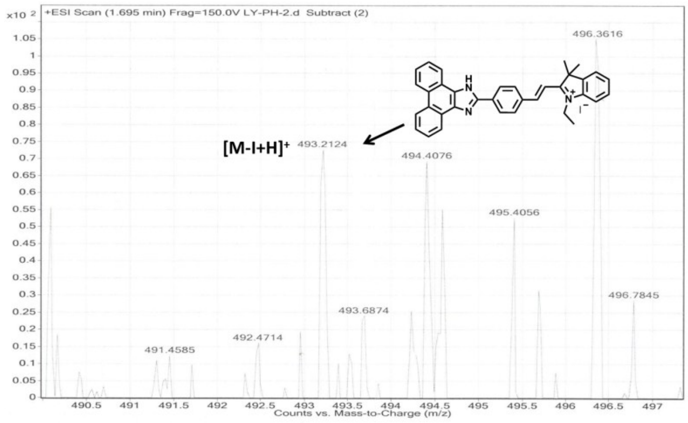


Fig. S18 HRMS spectrum of the compound **PID**.

References

1. S. Damavandi, *Heterocycl. Commun.*, 2011, **17**, 79.
2. J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu and B. Z. Tang, *Chem. Commun.*, 2001, 1740.
3. Y. Hong, J. W. Y. Lam and B. Z. Tang, *Chem. Commun.*, 2009, 4332.
4. J. Liu, J. W. Y. Lam and B. Z. Tang, *J. Inorg. Organomet. Polym. Mater.*, 2009, **19**, 249.
5. Y. Hong, J. W. Y. Lam and B. Z. Tang, *Chem. Soc. Rev.*, 2011, **40**, 5361.
6. B. Chen, H. Nie, P. Lu, J. Zhou, A. Qin, H. Qiu, Z. Zhao and B. Z. Tang, *Chem. Commun.*, 2014, **50**, 450.
7. M. Wang, G. Zhang, D. Zhang, D. Zhu and B. Z. Tang, *J. Mater. Chem. B.*, 2010, **20**, 1858.
8. J. Mei, J. Tong, J. Wang, A. Qin, J. Z. Sun and B. Z. Tang, *J. Mater. Chem.*, 2012, **22**, 17063.
9. H. Shi, J. Liu, J. Geng, B. Z. Tang and B. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 9569.
10. H. Tong, Y. Hong, Y. Dong, M. Haussler, Z. Li, J. W. Y. Lam, Y. Dong, H. H. Y. Sung, I. D. Williams and B. Z. Tang, *J. Phys. Chem. B.*, 2007, **111**, 11817.
11. W. Wang, T. Lin, M. Wang, T. Liu, L. Ren, D. Chen and S. Huang, *J. Phys. Chem. B.*, 2010, **114**, 5983.
12. Y. Dong, J. W. Y. Lam, A. Qin, Z. Li, J. Liu, J. Sun, Y. P. Dong and B. Z. Tang, *Chem. Phys. Lett.*, 2007, **446**, 124.
13. W. Qin, Z. Yang, Y. Jiang, J. W. Y. Lam, G. Liang, H.S . Kwok and B. Z. Tang, *Chem. Mater.*, 2015, **27**, 3892.
14. Z. Yang, W. Qin, N. L. C. Leung, M. Arseneault, J. W. Y. Lam, G. Liang, H. H. Y. Sung, I. D. Williams and B. Z. Tang, *J. Mater. Chem. C.*, **2016**, **4**, 99.
15. X. Shen, Y. Wang, E. Zhao, W. Yuan, Y. Liu, P. Lu, A. Qin, Y. Ma, J.Sun and B. Z.

Tang, *J. Phys. Chem. C.*, 2013, **117**, 7334.

16. J. Li, Y. Li, C. Y. K. Chan, R. T. K. Kwok, H. Li, P. Zrazhevskiy, X. Gao, J. Sun, A.Qin and B. Z. Tang, *Angew. Chem. Int. Ed.*, 2014, **53**, 13518.
17. H. Wang, T. Lin , J. Ma and W. Wang, *Tetrahedron.*, 2014, **70**, 5046.
18. W. Zhang, W. Liu, P. Li, F. Huang, H. Wang and B. Tang, *Anal. Chem.*, 2015, **87**, 9825.
19. I. -T. Ho, K. -C. Haung and W. –S. Chung, *Chem. Asian J.*, 2011, **6**, 2738.
20. J. Geng, C. Goh, W. Qin, R. Liu, N. Tomczak, L. Ng, B. Z. Tang and B. Liu, *Chem. Commun.*, 2015, **51**, 13416.
21. Y. Cai, L. Li, Z. Wang, J. Sun, A. Qin and B. Z. Tang, *Chem. Commun.*, 2014, **50**, 8892.
22. Z. Zhu, C. W. T. Leung, X. Zhao, Y. Wang, J. Qian, B. Tang and S. He, *Scientific Reports.*, 2015, **5**, 15189.
23. X. Wang, H. Hu, W. Wang, A. Qin, J. Sun, B. Z. Tang, *Polym. Chem.* **2015**, *6*, 7958.
24. Y. Zhang, M. Jiang, G. Han, K. Zhao, B. Tang and K. S. Wong, *J. Phys. Chem. C.*, 2015, **119**, 27630.
25. Y. Gong, Yiren Zhang, W. Yuan, J. Sun and Y. Zhang, *J. Phys. Chem. C.*, 2014, **118**, 10998.