Supplementary Information (SI) for Analytical Methods.
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Supporting Information

A pyrene-derived fluorescence probe based on π-π stacking and excimer formation for DNA detection and intracellular visualization

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1. Experimental

Materials and General Instrumentation. Compounds 1-(4-bromophenyl)pyrene, iodomethane, ct-DNA, sodium phosphate, and calcium chloride were purchased from Aladdin (Shanghai, China). The solution of L-amino acid systems were purchased from Beijing Innochem Technology Co., Ltd., the specification is 10 mM in Water, and then diluted according to experimental needs. All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) purchased from Beyotime Institute of Biotechnology. 1H NMR and 13C NMR were measured on a Bruker AVANCE III HD 400MHz spectrometer. Chemical shifts (d values) were reported in ppm down field from internal Me4Si. High resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Melting points were recorded on a melting point apparatus (RY-2, Tianjin, China). UV-vis absorption spectra were obtained with UV-2550 (Shimadzu, Japan) spectrophotometer. A Hitachi F-4600 spectrophotometer (Tokyo, Japan) was used for fluorescence measurements with a 700 V PMT voltage. The pH values were reported by a Mettler Toledo Seven Excellence PH meter (Shanghai, China). The absorbance for MTT analysis was recorded on a microplate reader (PL-9602). The confocal microscopy imaging was used Olympus FV1000-IX81 inverted fluorescence microscope. Image processing was analyzed with Olympus software (FV1000-ASW).

Spectrophotometric and spectrofluorimetric titration The absorption and fluorescence emission spectra of L were measured in various solvents at room temperature. The concentration of L for the absorption and fluorescence emission spectra was set at 10 μM and 5 μM, respectively. The wavelength of maximum absorption was selected as the excitation wavelength (λex) for fluorescence emission measurements. Titration experiments were performed in water at room temperature. A freshly prepared L solution (5 μM) was titrated with DNA. After equilibration for 1 minute, UV/visible light or emission spectra were recorded. The excitation wavelength for L was set at 350 nm. The excitation and emission slit widths were set at 5 nm and 10 nm, respectively. The DNA titration experiments were performed as follows: Calf thoracic DNA (ct-DNA) was gradually added to a fixed concentration (5 μM) of L in water. The excitation wavelength (λex) for fluorescence measurements was set at 350 nm. Competition experiments were also performed in water. First, 5 μM L was incubated with ct-DNA (5 μM) for 30 min, and then interfering substances of the same concentration were added, and the fluorescence emission spectrum was recorded.

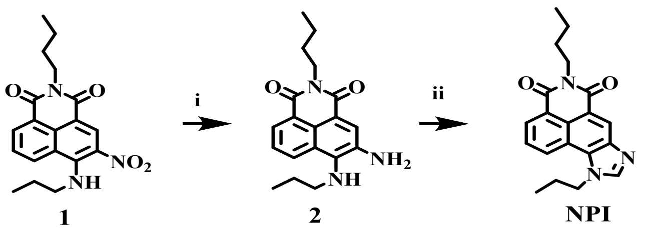
Determination of detection limit (LOD). [1]The standard deviation of the blank was determined by 11 measurements of the fluorescence emission spectra of L (5 μM). The linear relationship between the concentration of ct-DNA and the fluorescence intensity at 451 nm was determined by fitting a concentration-dependent fluorescence curve. The limit of detection (LOD) was calculated using the following equation:

$$\mathrm{LOD} = 3\sigma/k$$

Where σ was the standard deviation of the blank sample, k was the slope of the linear regression formula.

Cytotoxicity assay [2]. HeLa cells were seeded at 6×10^4 cells per well in 96-well plates and incubated for 24 h before treatment, followed by exposure to different concentrations (3, 6, 9, 12 and 15 μM) of **L** for 24 h, then washed with Dulbecco's Phosphate Buffered Saline (DPBS) and incubated with 20 μL MTT (5 mg/mL) solution for further 4 h. And then the cytotoxic effects of **L** was determined using MTT assays. The absorbance value at 350 nm was measured by a microplate reader. The cytotoxic effect (VR) of **L** was assessed using the following equation: $VR = A/A_0 \times 100\%$, where A and A_0 are the absorbance of the experimental group and control group, respectively. The assays were performed in six sets for each concentration.

Cell incubation and fluorescence imaging. HeLa cells were seeded in DMEM supplemented with 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) and 10% fetal bovine serum (FBS) and cultured at 37°C in a 5% CO_2 atmosphere. For a control group, cells were directly incubated with L (1 μM) at 37°C for 30 minutes. To image exogenous calcium ions, cells were incubated with various concentrations of Ca^{2+}(0, 100, and 200 μM) for 30 minutes, followed by the addition of L (1 μM) at 37°C for 30 minutes. Fluorescence imaging were performed by confocal fluorescence microscope with excitation wavelength at 405 nm and emission wavelength at the range of 422-435 nm.



Scheme S1 Synthesis of NPI. Reagents and conditions: i) $SnCl_2$, HCl (1 ml), EtOH, reflux, overnight; ii) formaldehyde, EtOH, room temperture, 2 h.

Synthesis of the naphthalimido-imidazole structure (NPI). The compound 2 (40 mg, 0.12 mmol) and FA (370 mg, 12 mmol) were dissolved in EtOH (10 mL). Then, the mixture solution was stirred for 2 h at room temperture. The solvent was removed by evaporation under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2, CH_2Cl_2/EtOH, gradient) to obtain **NPI** as a yellow solid (33 mg, yield 82%). ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 9.05 (s, 1H), 8.62 (d, 1H, J = 7.2 Hz), 8.48 (d, 1H, J = 8.4 Hz), 8.07 (s, 1H), 7.84 (t, 1H, J = 7.8 Hz), 4.61 (t, 1H, J = 7.0 Hz), 4.20 (t, 1H, J = 7.4 Hz), 2.12-2.06 (m, 2H), 1.78-1.70 (m, 2H), 1.51-1.42 (m, 2H), 1.08 (t, 3H, J = 7.4 Hz), 0.98 (t, 3H, J = 7.4 Hz); 164.48, 164.29, 145.14, 141.95, 131.67, 128.86, 127.36, 126.69, 126.31, 126.14, 123.87, 120.32, 118.07, 49.07, 40.45, 30.22, 22.13, 20.45, 13.89, 11.13.

2. Additional data and spectrum

Table S1 Photophysical properties of **L** in different solvents

Solvents	λ_{abs} (nm)	ε ($M^{-1}cm^{-1}$)	Pl_{max} (nm)	Θ	Stokes shife (nm)
DCM	347	34200	524/475	0.255	177/128
1.4-Dioxance	345	34600	450	0.107	105
MeCN	344	34700	504	0.106	160
EtOH	344	34700	552/440	0.035	208/96
DMSO	350	33500	512	0.134	162
DMF	348	33300	495	0.106	147
MeOH	343	34400	557/439	0.028	214/96
H_2O	352	19500	451	0.098	99

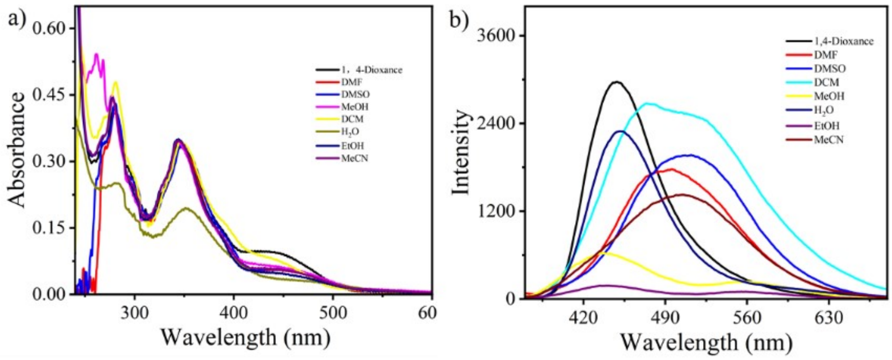


Fig. S1. a) UV/vis absorption (10 μM) and b) fluorescence emission (5 μM) spectra of the probe L in different solvents. λex = 350 nm.

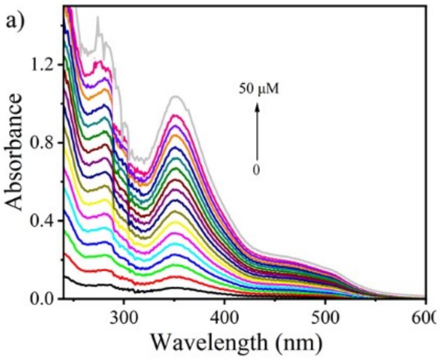


Fig. S2. a) The absorption spectrum of L with the increased concentration (0-50 μM) in water; b)The absorption spectrum of L with the increased concentration (0-25 μM) in ethanol. The absorbance of L at 352 nm in water and at 345 nm in ethanol enhanced along with the increased concentration of L.

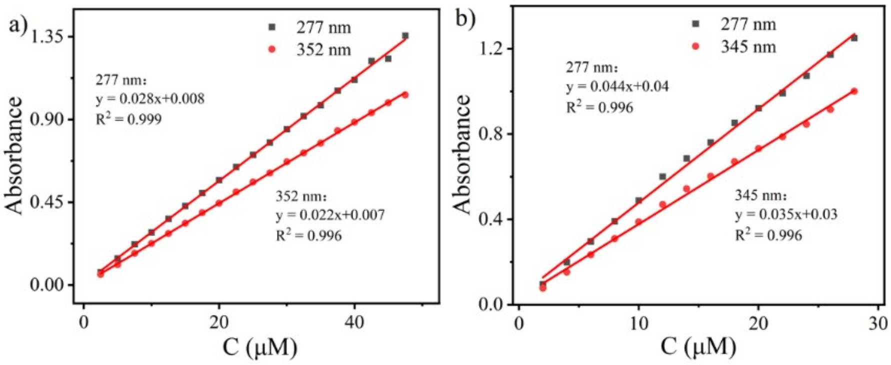


Fig. S3. a) Linear fitting plots of UV/vis absorption intensity of L with increasing concentration in water, at wavelengths of 277 nm and 352 nm; b) Linear fitting plots of UV/vis absorption intensity of L with increasing concentration in ethanol, at wavelengths of 277 nm and 345 nm.

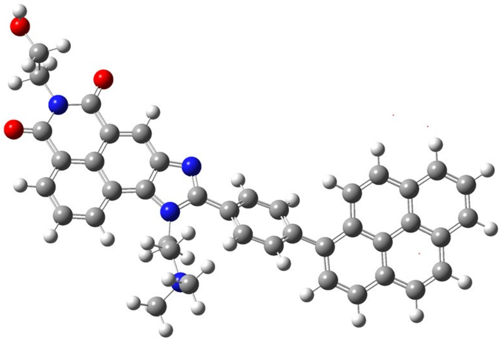


Fig. S4. The theoretically optimized geometry of probe L

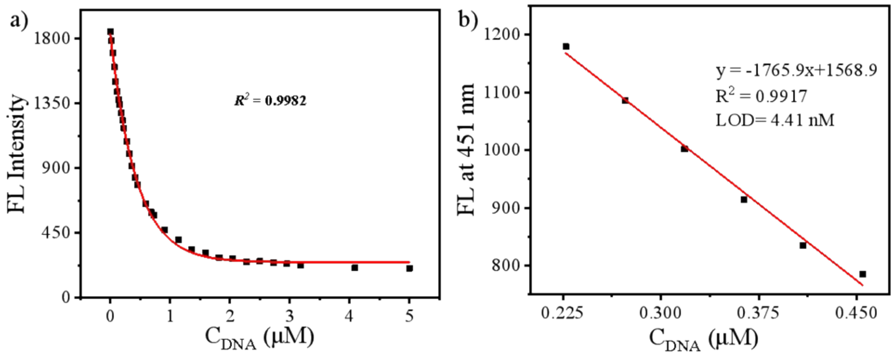


Fig. S5 a) The fitting curve of fluorescence intensity (451 nm) of probe L as a function of DNA concentration; b) the linear fitting curve of fluorescence intensity (451 nm) of L versusin relation to DNA concentration within the low - concentration DNA range (0-0.5 μM).

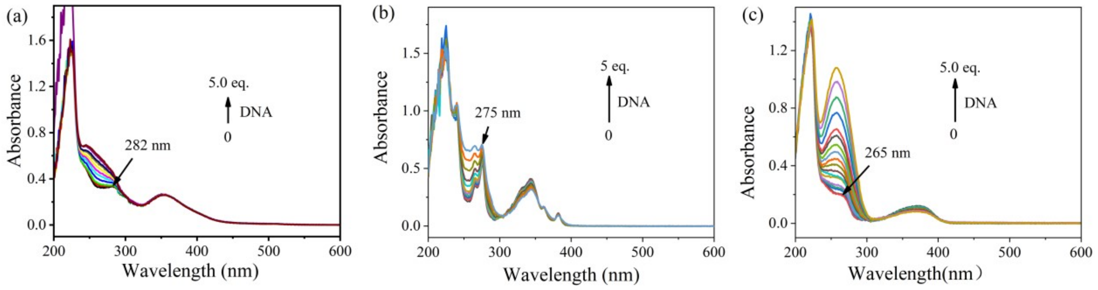


Fig. S6 the absorbance spectra of L (a, 5 μM), pyrene (b, 5 μM) and NPI (c, 5 μM) in aqueous solution in the presence of different concentrations of DNA (0-5.0 equiv.).

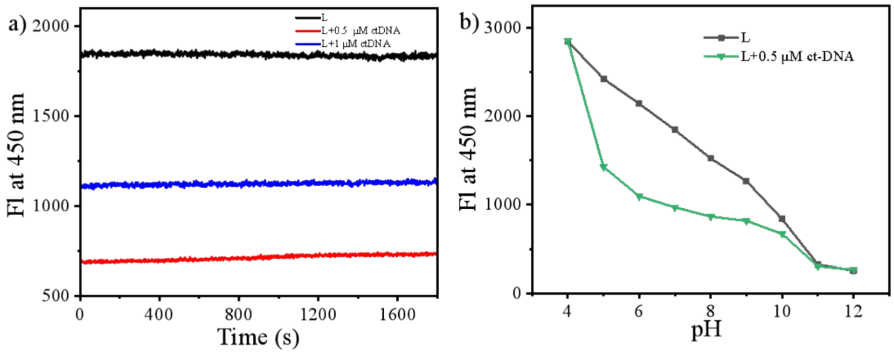


Fig. S7 a) The time-dependent fluorescence signals of **L** (5 μM) with addition of different concentrations of DNA (0, 0.5 and 1 μM) in water. b) The pH-dependent fluorescence signals of **L** (5 μM) in the absence and presence of DNA (0.5 μM). λ_{ex} = 350 nm, λ_{em} = 451 nm.

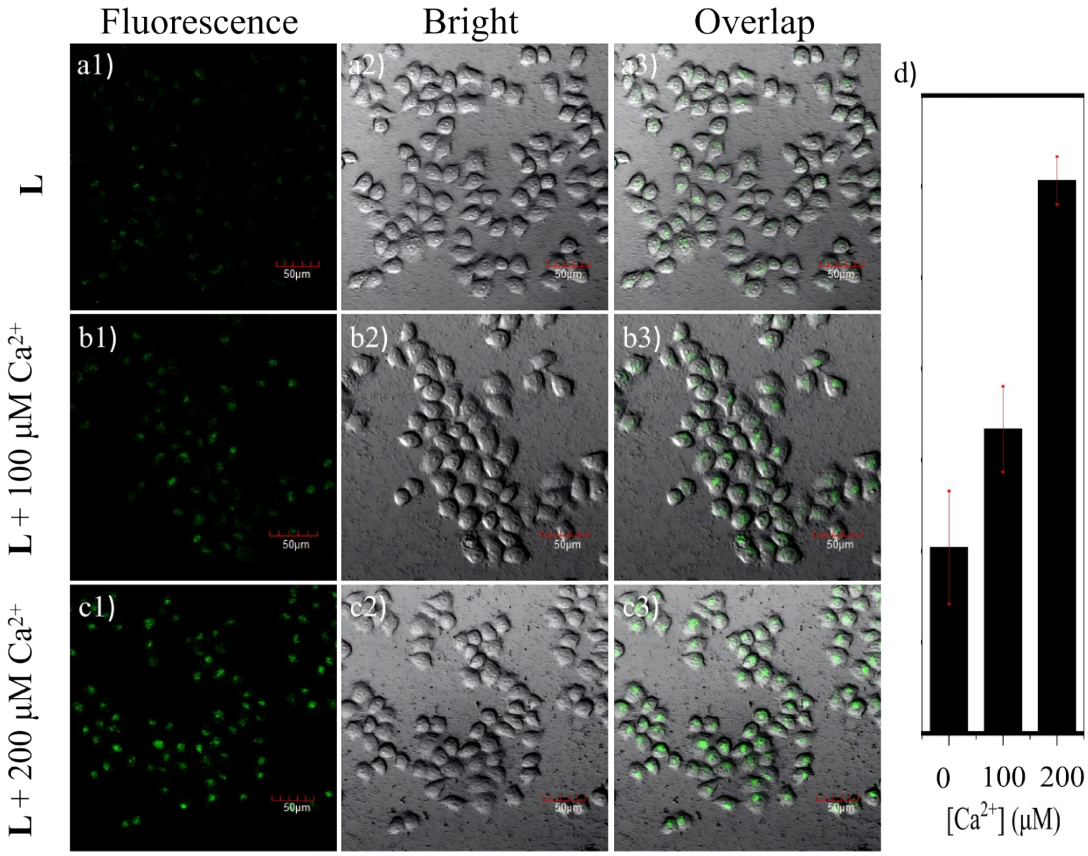


Fig. S8 Fluorescence images of HeLa cells that were first incubated with L (1 µM) for 20 min, followed by incubation with different concentrations of Ca^{2+} (0, 100, and 200 µM) for 15 min. (c) The IOD of the nucleus and cytoplasm in the HeLa cells treated with L (1 µM, a1) and L + Ca^{2+} (b1, c1).Incubation temperature: 37 °C; λex = 405 nm; λem = 422-435 nm. Scale bars: 50 µm.

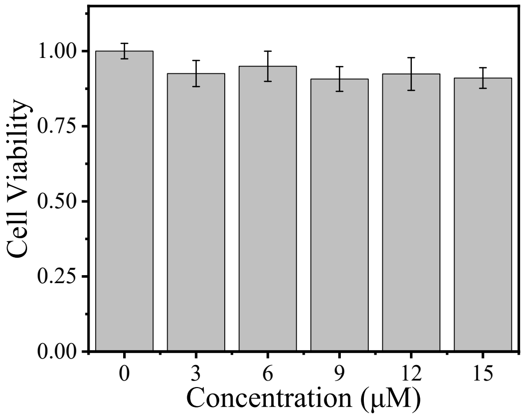


Fig. S9 Cytotoxicity of L in HeLa cells. The cells were incubated with L at corresponding concentrations (0 µM, 3 µM, 6 µM, 9 µM, 12 µM, 15 µM) for 24 h.

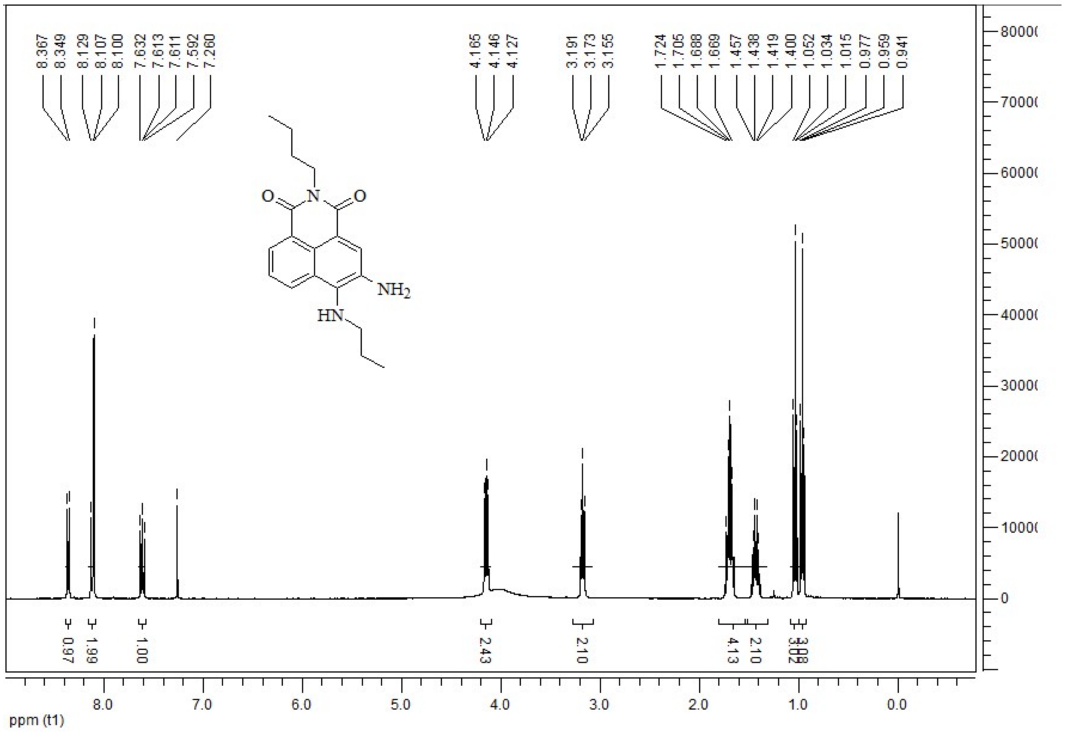


Fig. S10. ^{1}H NMR of the probe **2**

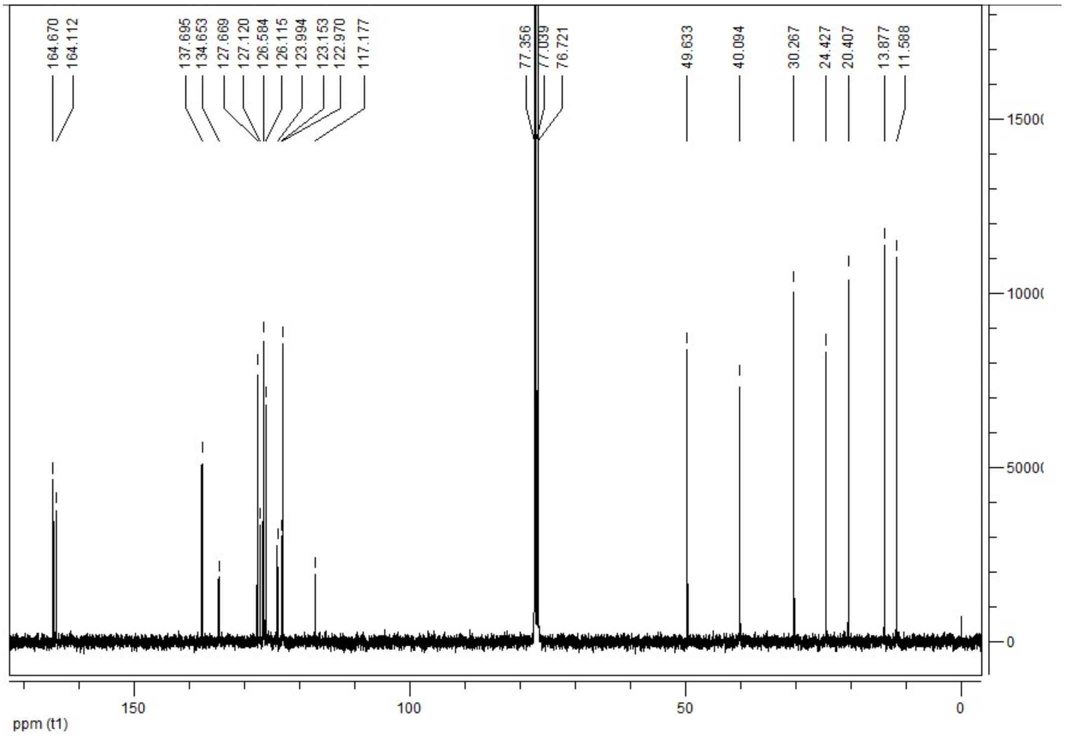


Fig. S11. ^{13}C NMR of **2**

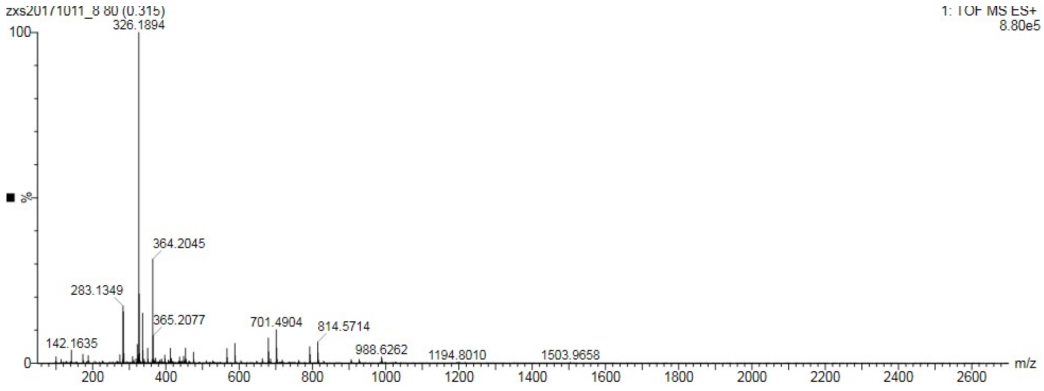


Fig. S12. HRMS spectrum of **2**

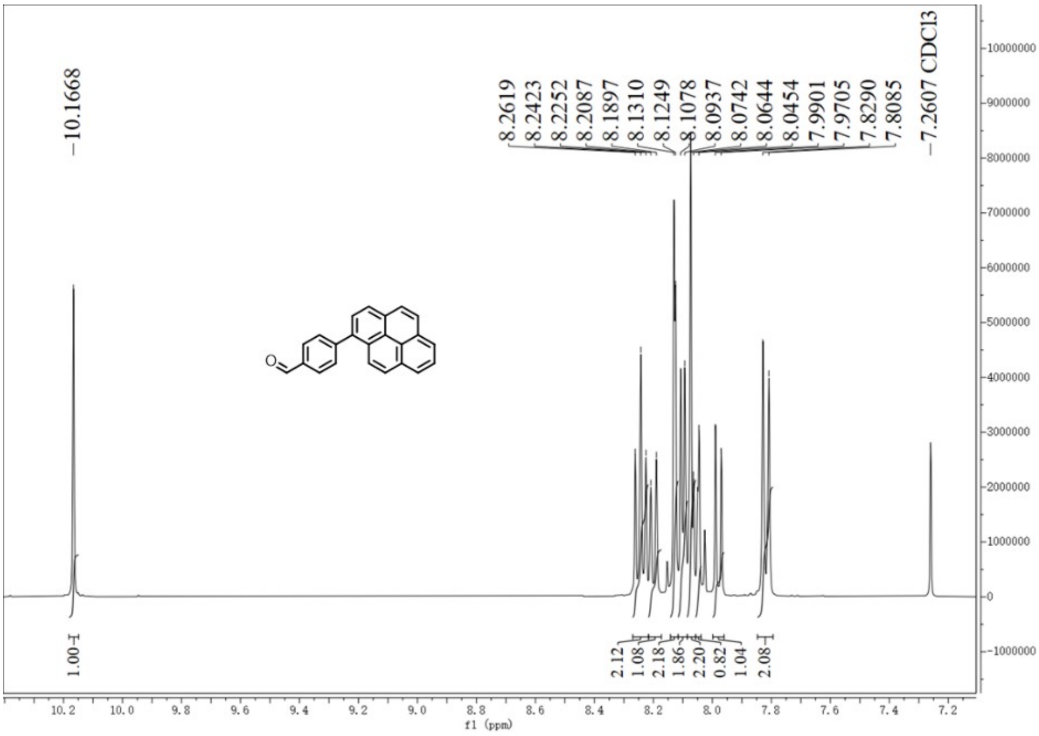


Fig. S13. ^{1}H NMR of **4.**

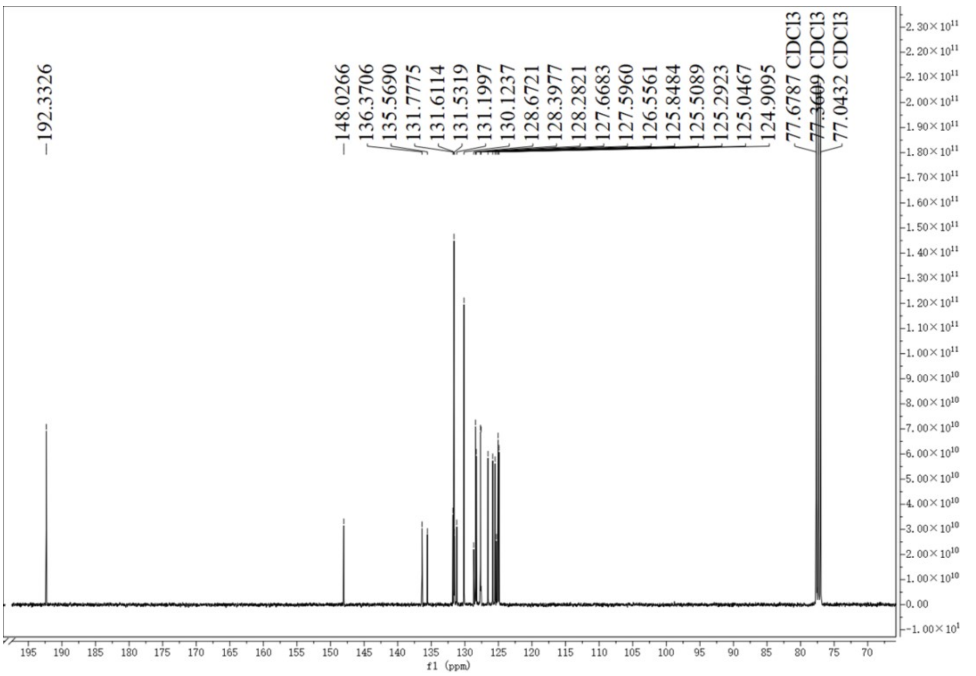


Fig. S14. ^{13}C NMR of **4.**

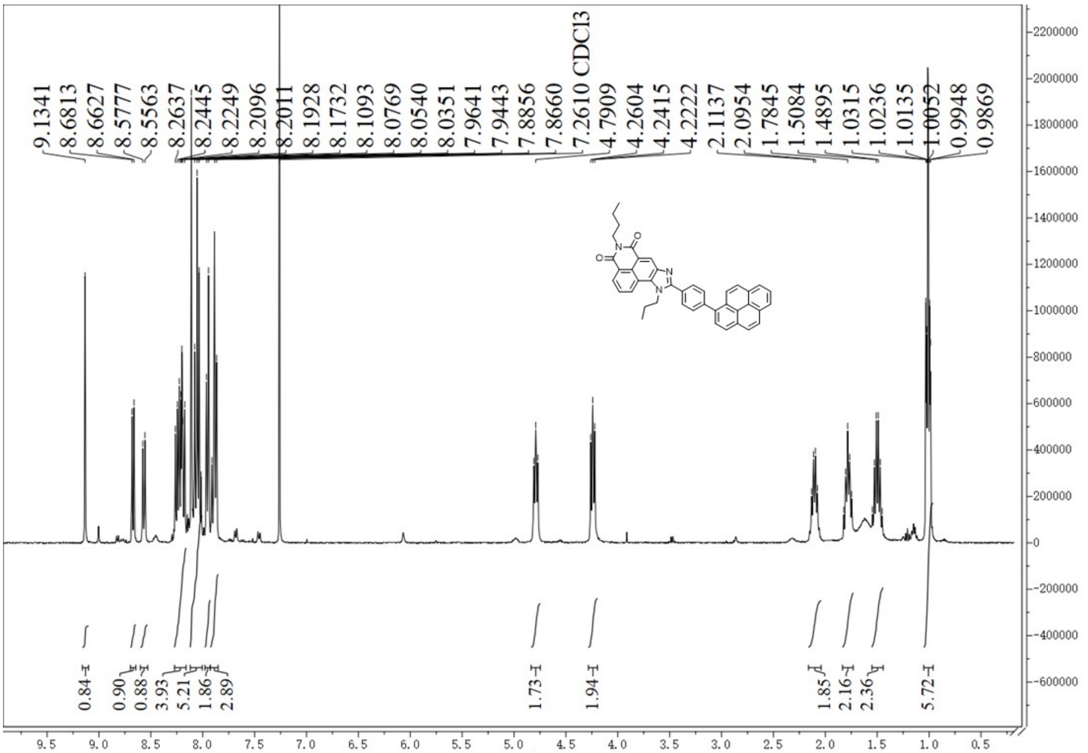


Fig. S15. ^{1}H NMR of **L.**

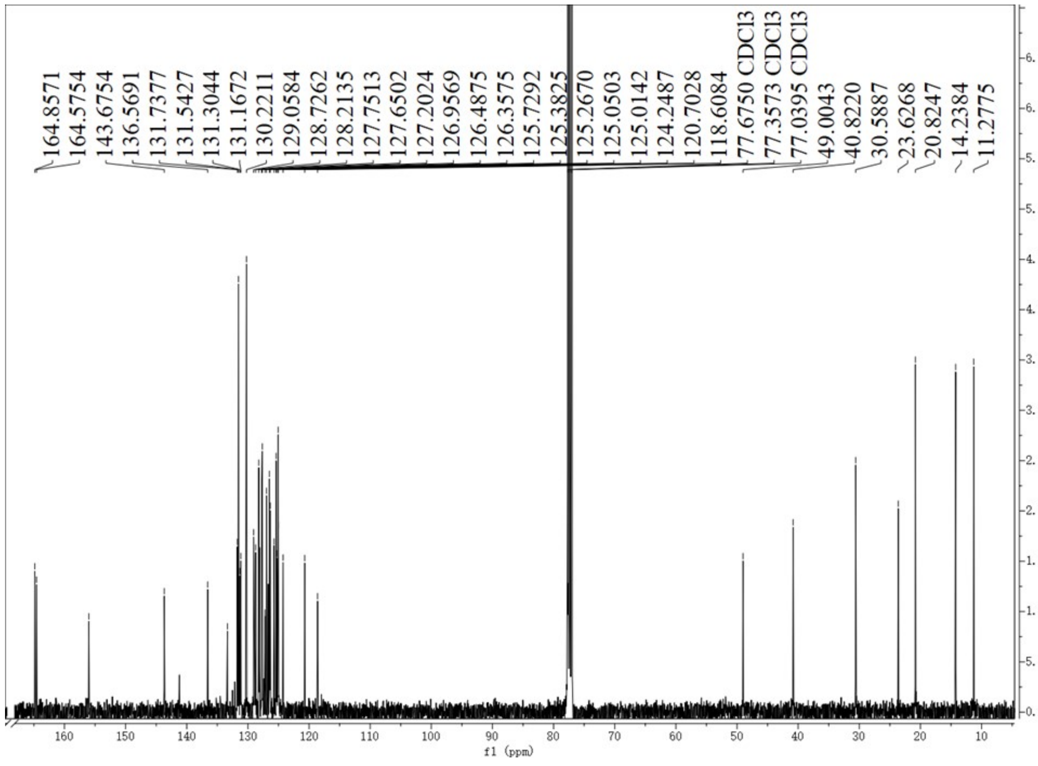


Fig. S16. ^{13}C NMR of **L.**

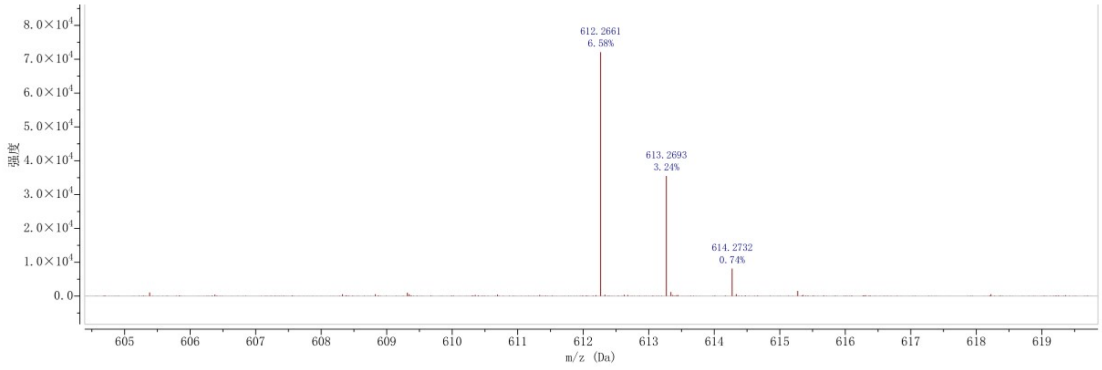


Fig. S17. HRMS spectrum of **L**

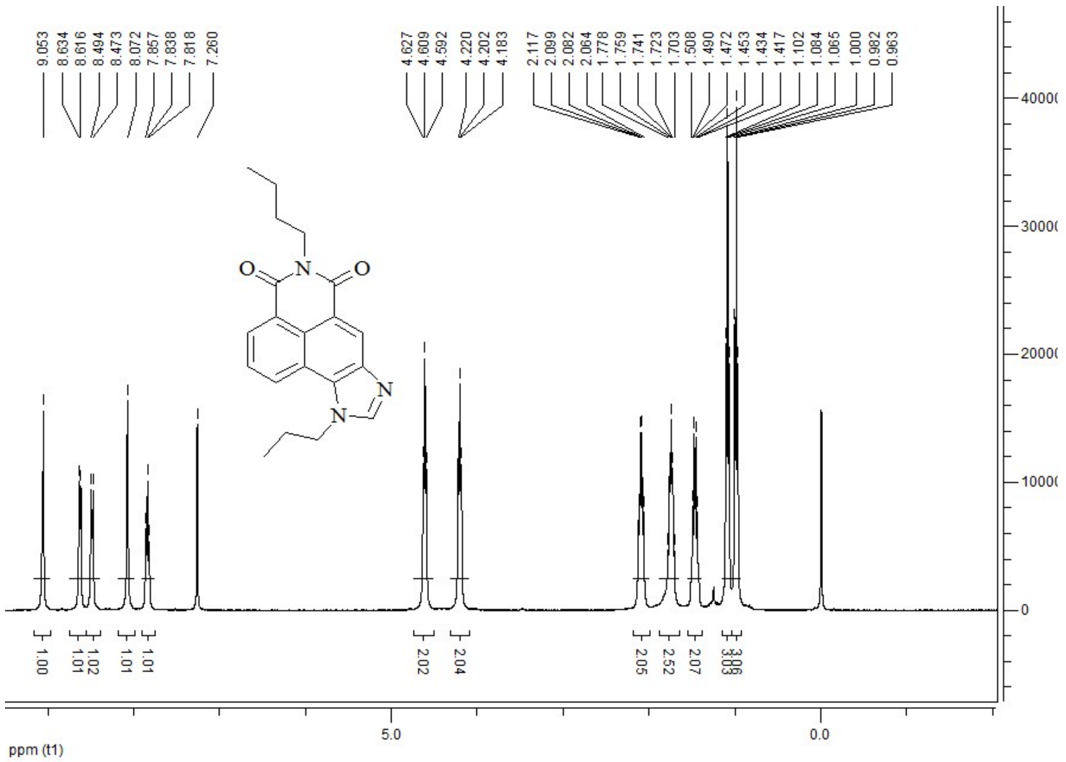


Fig. S18. ^{1}H NMR of **NPI**

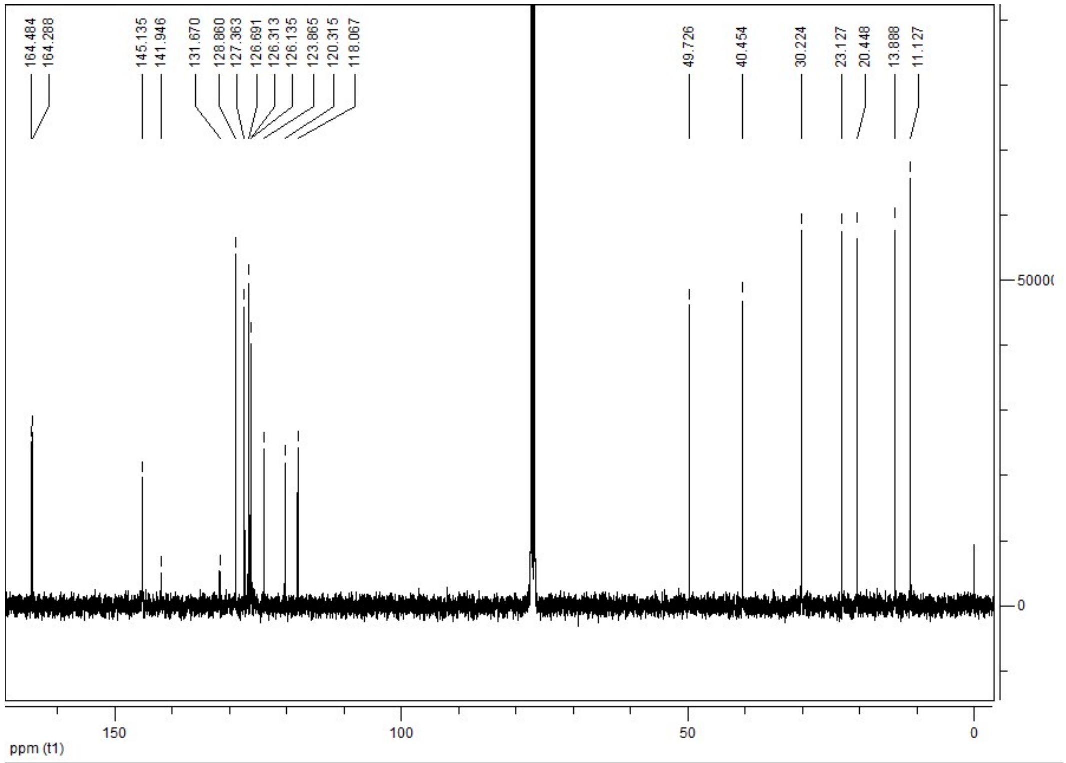


Fig. S19. ^{13}C NMR of **NPI**.

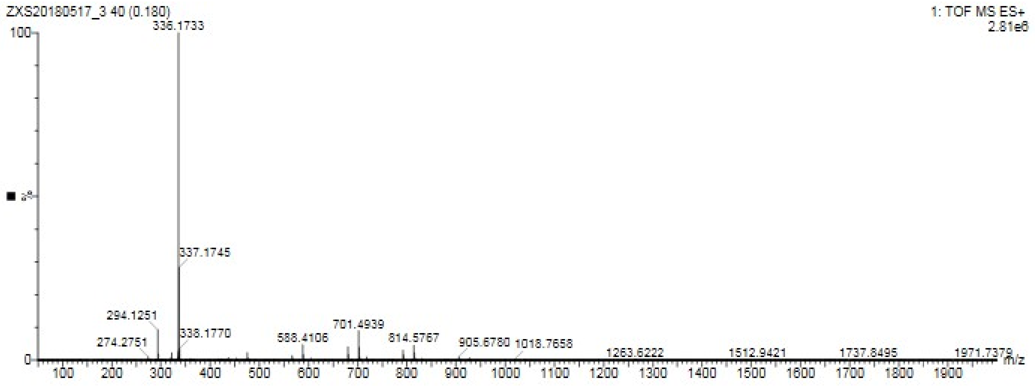


Fig. S20. HRMS spectrum of **NPI**

2. References

[1] G. Long, J. Winefordner, Limit of detection a closer look at the IUPAC definition, Anal. Chem. 55 (1983) 712-724.

[2] T.Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55-63.

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