Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Donor-acceptor strategy to construct near infrared AIEgens for cell imaging

Xinyu Yang,[a,†] Chunbin Li,[b,†] Lingxiu Liu,[b] Hongge Zhang,[c] Hai-Tao Feng,[c] Yongdong Li,[a,*] Guoyu Jiang[b,*] and Jianguo Wang[b]

[a]*Key Laboratory of Organo-Pharmaceutical Chemistry, Gannan Normal University, Ganzhou 341000, P. R. China.*
[b]*College of Chemistry and Chemical Engineering, Inner Mongolia University, Hohhot 010021, P. R. China. *E-mail: jiangguiyu@mail.ipc.ac.cn.*
[c]*AIE Research Center, Shaanxi Key Laboratory of Phytochemistry, College of Chemistry and Chemical Engineering, Baoji University of Arts and Sciences, Baoji 721013, P. R. China.*

Experiment section:

Materials and instrumentation.

All chemicals and reagents were purchased from Titan and used without any further purification, unless otherwise stated. Phosphate buffered PBS (PBS, pH 7.4) were purchased from Sigma-Aldrich. Dulbecco's Modified Eagle's Medium (DMEM) medium, fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco. Double distilled water was supplied by Milli-Q Plus System (Millipore Corporation, Bedford, USA).

^{1}H and ^{13}C NMR spectra were recorded with a Bruker ARX 500 NMR spectrometer using tetramethylsilane (TMS) as a reference at room temperature. High resolution mass spectra were collected on a Waters G2-Xs QTOF mass spectrometer. Absorption spectra were measured on a SHIMADZU UV-2600i spectrophotometer. Steady-state photoluminescence (PL) spectra were recorded on a HITACHI F-4700 spectrophotometer. Density functional theory (DFT) and time-dependent density function theory (TD-DFT) calculations were carried out by the B3LYP/6-311G(d) using Gaussian 09 package. Cellular imaging experiments were performed with confocal laser scanning microscope (LSM880, ZEISS, Germany) equipped with Argon, red HeNe, and green HeNe lasers.

Cell cultures

The HeLa cells were cultured in DMEM (containing 10% heat-inactivated FBS, 100 $mg{\cdot}mL^{-1}$ penicillin and 100 $mg{\cdot}mL^{-1}$ streptomycin) at 37 ^{o}C in a humidified incubator with 5% CO_2. Before the experiments, the cells were pre-cultured until confluence was reached.

Cell viability

Cell viability was determined by using MTT assay which is based on the reduction of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, yellow in color) into formazan (blue color) by mitochondrial succinate dehydrogenase. Dispense 100 μL of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 h at 37 °C in a humidified incubator with 5% CO_2. Add 10 μL of various concentrations

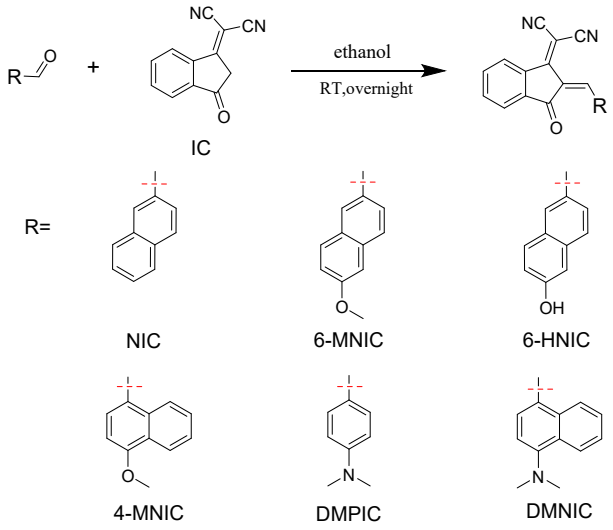
of DMNIC into the culture media in the plate. After incubating the plate for 20 h in the incubator, the cells were exposed to 660 nm laser irradiation (0.1 W cm^{-2}, DMNIC) or white light (10 mW cm^{-2}, other AIEgens except DMNIC) for 30 min. Meanwhile, the AIEgens-incubated cells without light irradiation were also conducted for the dark cytotoxicity study. After further incubation for 4 h, the medium was exchanged with fresh medium (100 μL) and 20 μg/mL MTT was then added. Medium was removed after the incubation period of 4 hours followed by the addition of 100 μL of DMSO to dissolve the formazan crystals. Absorbance was taken at 595 nm by an ELISA Plate Reader (Biotek Synergy HT). Untreated cells were taken as control. All the experiments were performed in triplicate. Cell viability was determined by using given formula:

$$\text{Cell viability (\%)} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated\ cells} \quad (1)$$

Cell treatment and cell imaging

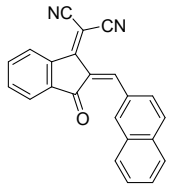
For cell imaging, the HeLa cells were incubated with 100 μM DMNIC for 7 h at 37 °C, then the cells were washed with PBS three times. The imaging was acquired using a Zeiss LSM 880 laser scanning microscopy. A 543 nm laser was used as the light source and emission was collected from 570 to 900 nm.

Synthesis and characterization



Scheme S1 Synthetic route of the six AIEgens.

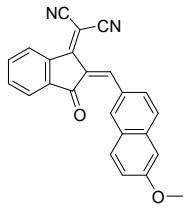
Synthesis of NIC



Chemical Formula: $C_{23}H_{12}N_2O$
Molecular Weight: 332.36200

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (125 mg, 0.64 mmol) and 2-naphthaldehyde (100 mg, 0.64 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. Then the solvent was evaporated and the residue was subjected to column chromatography with PE: EA= 5: 1 (v:v) as the eluent. The crude product was recrystallization with DCM and n-hexane, and an orange-red solid was obtained (254 mg, yield: 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.73 (d, J = 7.9 Hz, 1H), 8.67 (s, 1H), 8.24 (dd, J = 8.7, 1.9 Hz, 1H), 7.97 (t, J = 8.0 Hz, 2H), 7.92 – 7.78 (m, 4H), 7.62 (t, J = 7.5 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 186.37, 161.61, 147.89, 139.71, 137.39, 136.61, 135.64, 135.47, 135.06, 132.62, 130.15, 129.81, 129.67, 129.30, 128.96, 128.02, 127.77, 126.94, 125.37, 124.42, 114.09, 113.80, 72.49. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{23}H_{13}N_2O^+$: 333.1028; found: 333.1027.

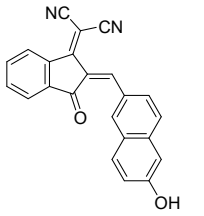
Synthesis of 6-MNIC.



Chemical Formula: $C_{24}H_{14}N_2O_2$
Molecular Weight: 362.38800

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (105 mg, 0.54 mmol) and 6-methoxy-2-naphthaldehyde (100 mg, 0.54 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. Then the solvent was evaporated and the residue was subjected to column chromatography with DCM as the eluent. The solvent was removed by vacuum distillation. The crude product was recrystallization with DCM and n-hexane as a reddish brown solid (246 mg, yield: 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.77 (s, 1H), 8.74 (d, J = 7.8 Hz, 1H), 8.71 (s, 1H), 8.34 (dd, J = 8.7, 1.9 Hz, 1H), 7.98 (d, J = 7.3 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.85 – 7.79 (m, 3H), 7.23 (dd, J = 8.9, 2.4 Hz, 1H), 7.19 (s, 1H), 4.00 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 186.66, 162.13, 160.86, 148.33, 139.71, 137.66, 137.42, 137.29, 135.45, 134.87, 131.73, 130.13, 128.43, 128.26, 128.14, 126.90, 125.29, 124.27, 119.90, 114.32, 114.05, 106.01, 71.65, 55.56. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{24}H_{15}N_2O_2^+$: 363.1134; found: 363.1132.

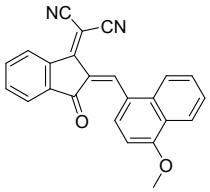
Synthesis of 6-HNIC.



Chemical Formula: $C_{23}H_{12}N_2O_2$
Molecular Weight: 348.36100

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (113 mg, 0.58 mmol) and 6-hydroxy-2-naphthaldehyde (100 mg, 0.58 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. After the reaction, solids were precipitated directly, and the pure product was obtained by filtration as a brown solid (223 mg, yield: 85%). ^{1}H NMR (500 MHz, DMSO) δ: 8.63 (s, 1H), 8.56 (s, 1H), 8.51 (d, J = 7.9 Hz, 1H), 8.22 (d, J = 8.9 Hz, 1H), 7.97 (t, J = 8.2 Hz, 2H), 7.90 (d, J = 8.1 Hz, 2H), 7.78 (d, J = 9.4 Hz, 1H), 7.21 – 7.17 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 186.35, 162.72, 159.43, 147.53, 139.54, 137.75, 137.43, 136.26, 135.72, 132.48, 129.90, 127.64, 127.16, 126.47, 124.81, 124.43, 120.11, 115.00, 114.76, 109.54, 99.99, 71.31. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{23}H_{13}N_2O_2^+$: 349.0977; found: 349.0974.

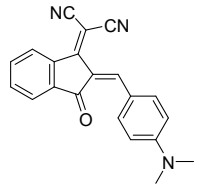
Synthesis of 4-MNIC.



Chemical Formula: $C_{24}H_{14}N_2O_2$
Molecular Weight: 362.38800

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (105 mg, 0.54 mmol) and 4-methoxy-1-naphthaldehyde (100 mg, 0.54 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. After the reaction, solids were precipitated, and the pure product was obtained by filtration as a brown solid (236 mg, yield: 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.43 (s, 1H), 8.76 (d, J = 7.9 Hz, 1H), 8.63 (d, J = 8.4 Hz, 1H), 8.38 (d, J = 8.3 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 7.84 (t, J = 7.3 Hz, 1H), 7.78 (t, J = 7.4 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 4.17 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 186.25, 162.37, 161.31, 144.81, 139.55, 137.36, 136.74, 135.32, 134.72, 134.05, 129.09, 127.90, 126.03, 125.31, 125.20, 124.18, 123.32, 123.22, 121.37, 114.46, 103.60, 71.08, 56.13. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{24}H_{15}N_2O_2^+$: 363.1134; found: 363.1134.

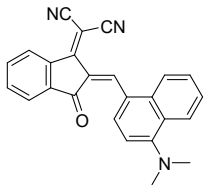
Synthesis of DMPIC.



Chemical Formula: $C_{21}H_{15}N_3O$
Molecular Weight: 325.37100

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (130 mg, 0.67 mmol) and 4-(dimethylamino) benzaldehyde (100 mg, 0.67 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. After the reaction, solids were precipitated, and the pure product was obtained by filtration as a dark brown solid (272 mg, yield: 80%). ^{1}H NMR (500 MHz, DMSO) δ: 8.46 (d, J = 7.9 Hz, 1H), 8.33 – 8.27 (m, 3H), 7.90 – 7.87 (m, 1H), 7.82 (br, 2H), 6.92 (d, J = 9.0 Hz, 2H), 3.21 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 187.35, 163.45, 154.67, 148.16, 139.61, 139.10, 137.31, 134.39, 133.87, 124.74, 123.41, 122.54, 121.92, 115.44, 115.26, 111.52, 67.24, 40.24. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{21}H_{16}N_3O^+$: 326.1293; found: 326.1291.

Synthesis of DMNIC.



Chemical Formula: $C_{25}H_{17}N_3O$
Molecular Weight: 375.43100

2-(3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile (97 mg, 0.5 mmol) and 4-(dimethylamino)-1-naphthaldehyde (100 mg, 0.5 mmol) were dissolved in anhydrous ethanol (6 mL). The reaction mixture was stirred overnight at room temperature. After the reaction, solids were precipitated, and the pure product was obtained by filtration as a dark green solid (250 mg, yield: 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.36 (s, 1H), 8.69 (d, J = 7.7 Hz, 1H), 8.64 (d, J = 8.4 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 5.5 Hz, 1H), 7.76 (t, J = 7.7 Hz, 1H), 7.71 (t, J = 7.4 Hz, 1H), 7.67 – 7.58 (m, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 3.17 (s, 6H). ^{13}C NMR (126 MHz, CDCl3) δ: 186.42, 162.87, 158.32, 144.29, 139.52, 137.29, 135.41, 134.90, 134.28, 128.64, 126.33, 126.21, 125.78, 125.04, 124.91, 124.17, 123.84, 121.68, 115.10, 114.97, 111.57, 69.15, 44.53. HRMS (MALDI-TOF): m/z: $[M+H]^+$ calcd for $C_{25}H_{18}N_3O^+$: 376.1450; found: 376.1447.

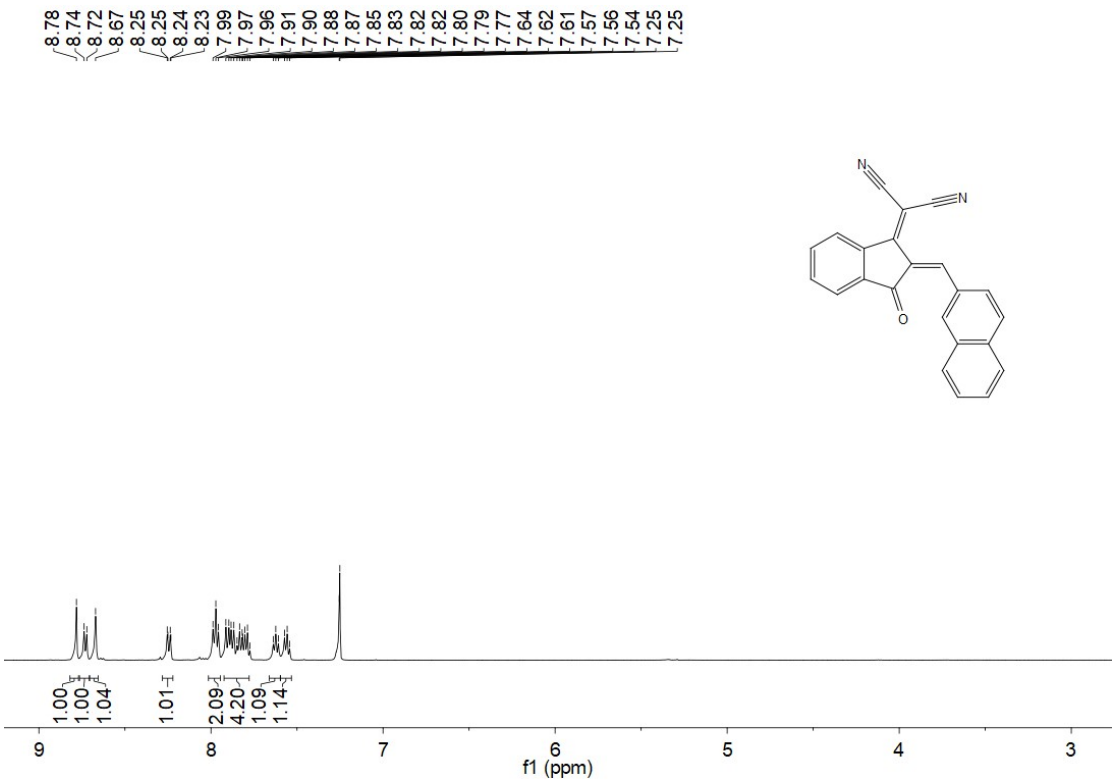


Figure S1. ^{1}H NMR spectrum of NIC in $CDCl_3$.

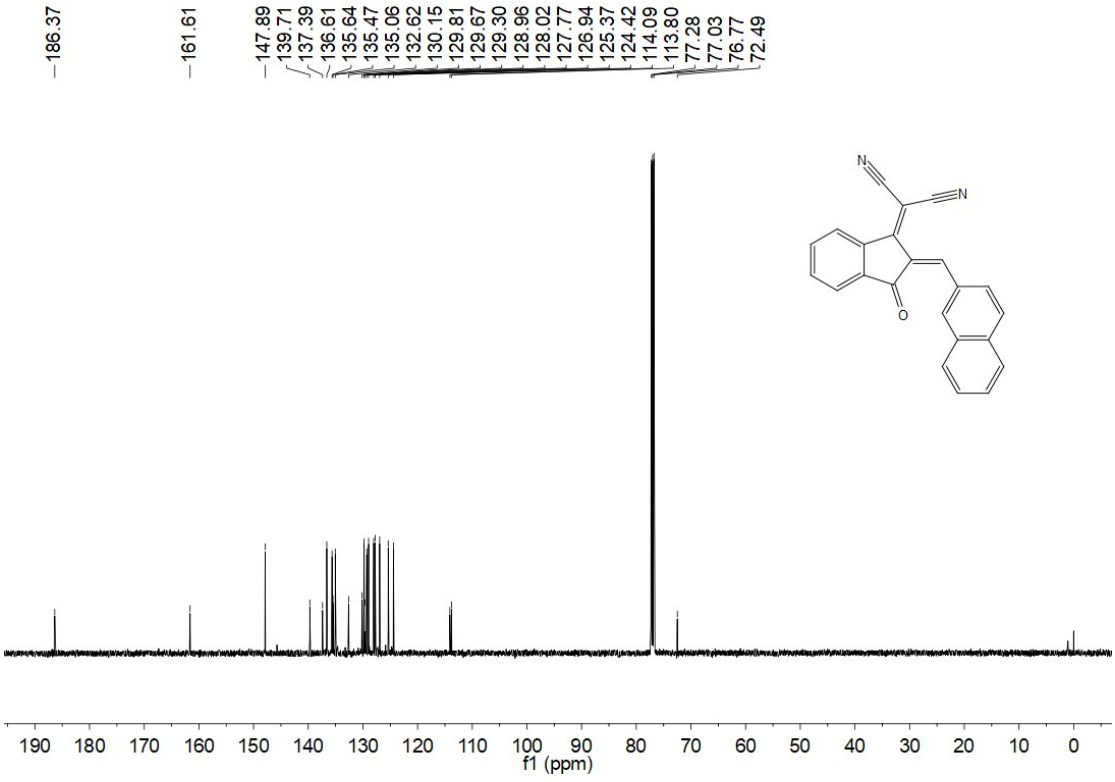


Figure S2. ^{13}C NMR spectrum of NIC in $CDCl_3$.

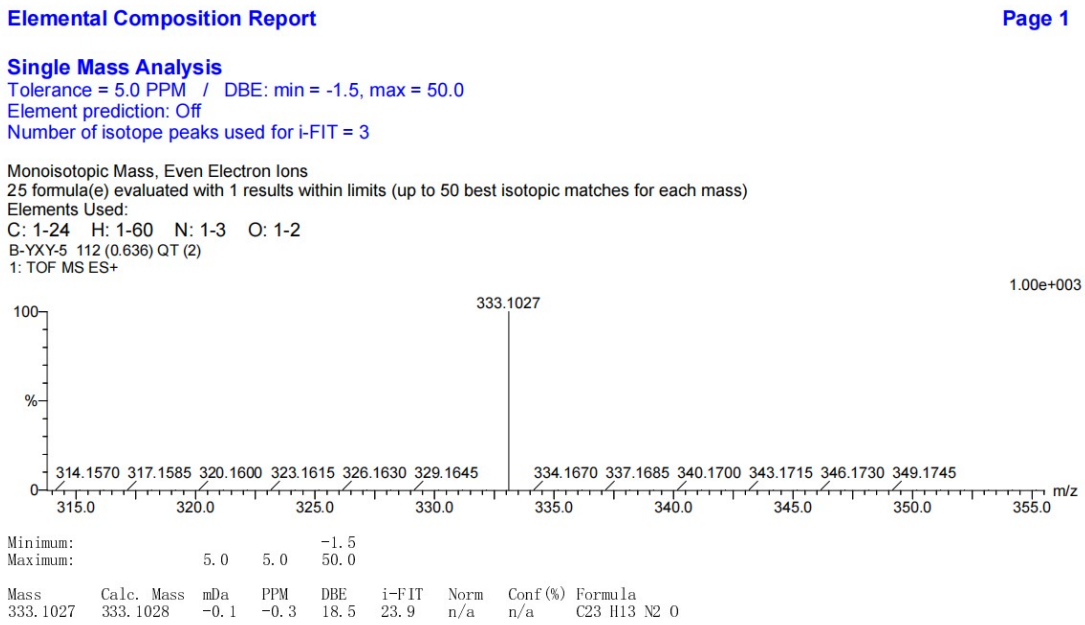


Figure S3. HRMS spectrum of **NIC**.

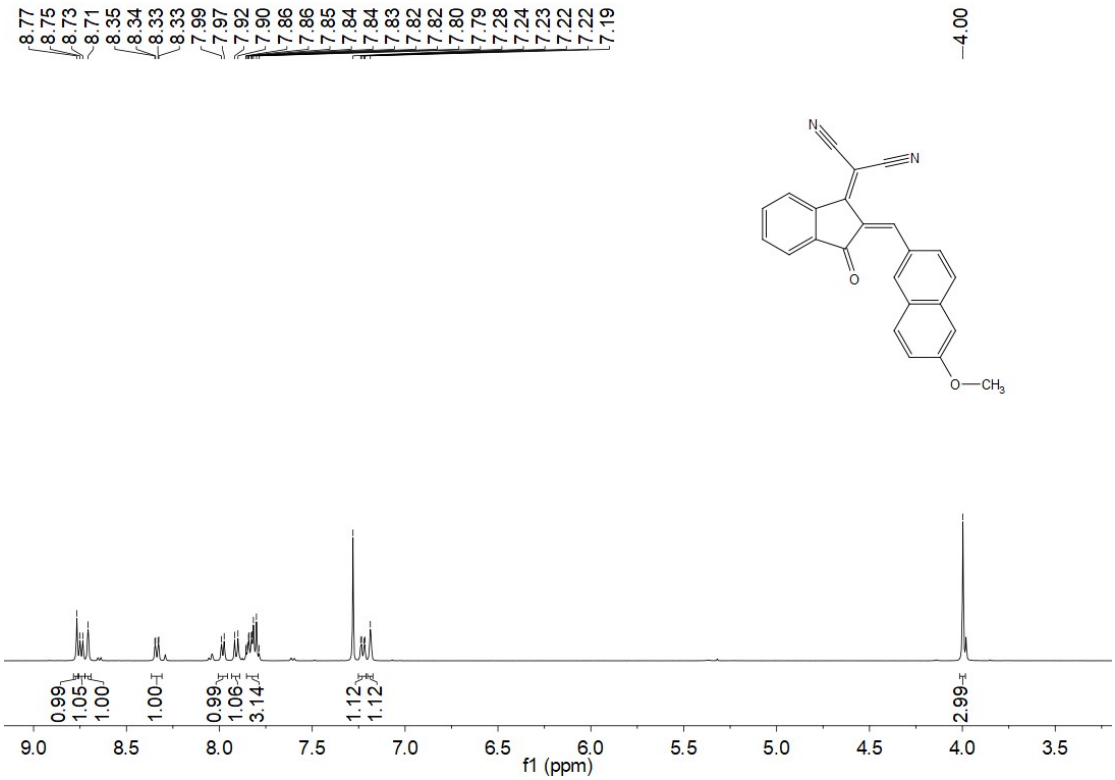


Figure S4. ^{1}H NMR spectrum of 6-MNIC in $CDCl_3$.

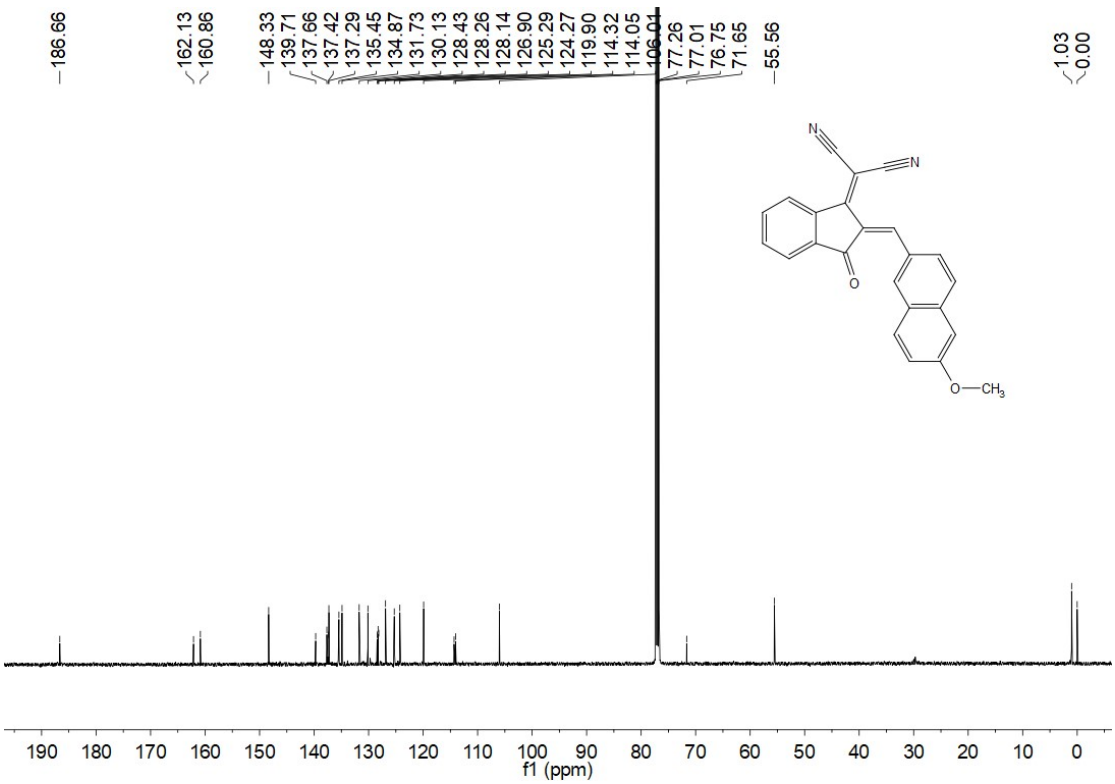


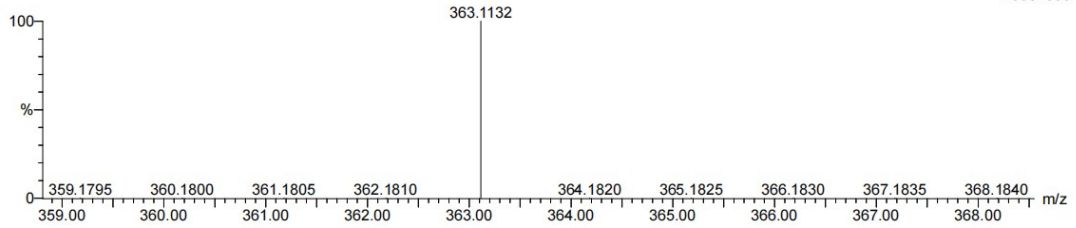
Figure S5. ^{13}C NMR spectrum of 6-MNIC in $CDCl_3$.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
12 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 1-24 H: 1-100 N: 1-2 O: 1-2
2
B-YXY-3 97 (0.558) QT (2)

1: TOF MS ES+
1.65e+003



Minimum:				-1.5				
Maximum:		5.0	5.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
363.1132	363.1134	-0.2	-0.6	18.5	25.3	n/a	n/a	C24 H15 N2 O2

Figure S6. HRMS spectrum of 6-MNIC.

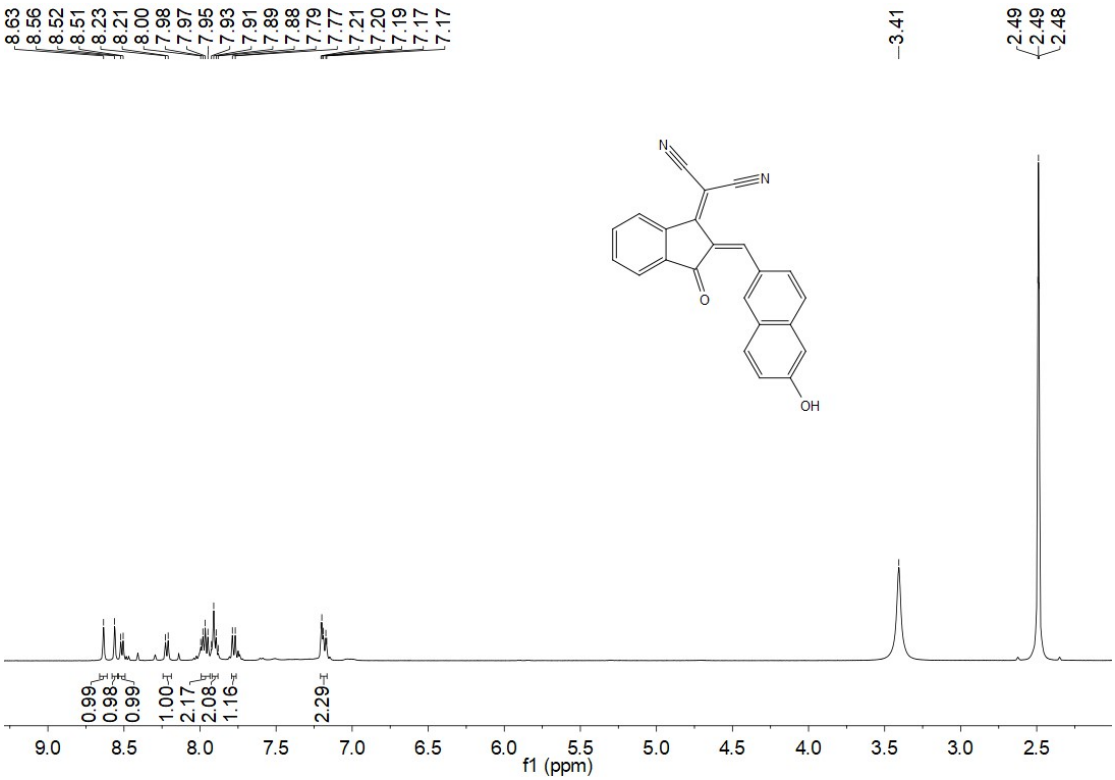


Figure S7. ^{1}H NMR spectrum of 6-HNIC in DMSO-d_6.

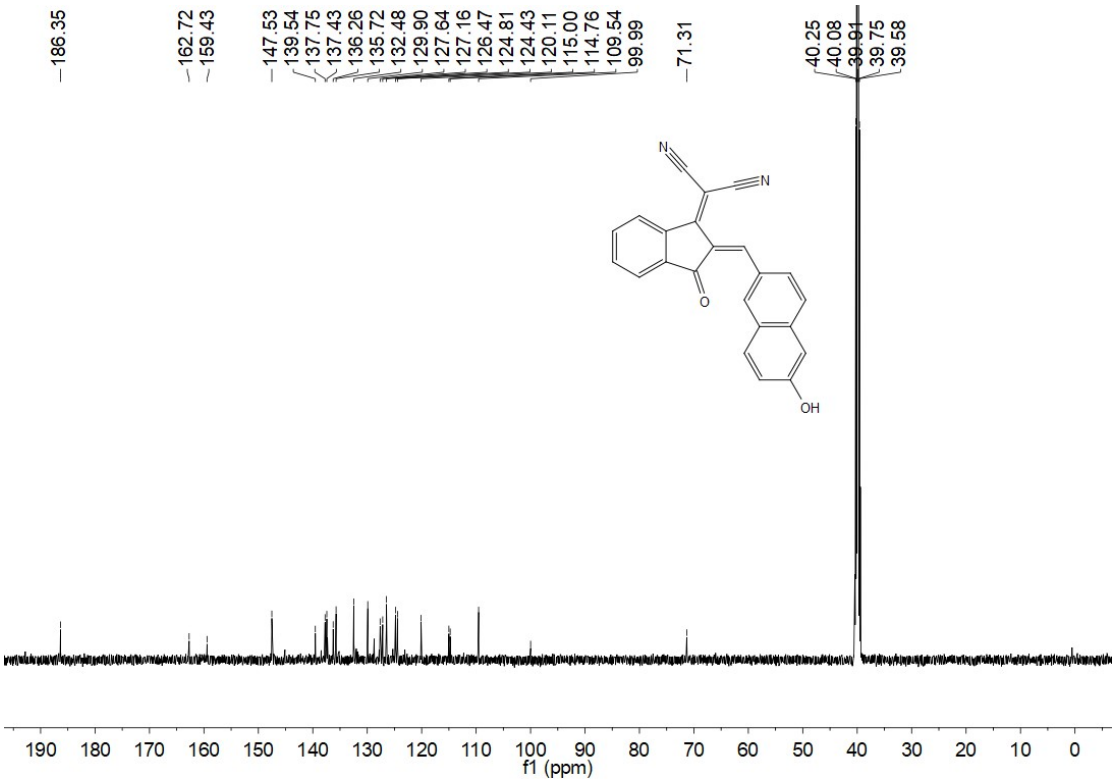


Figure S8. ^{13}C NMR spectrum of 6-HNIC in DMSO-d_6.

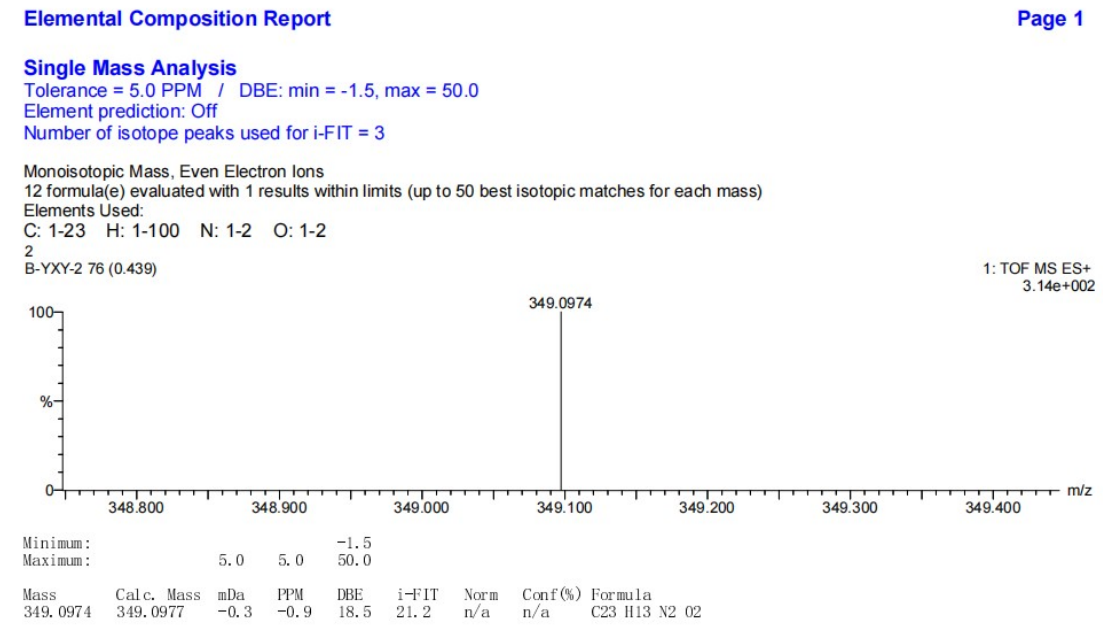


Figure S9. HRMS spectrum of 6-HNIC.

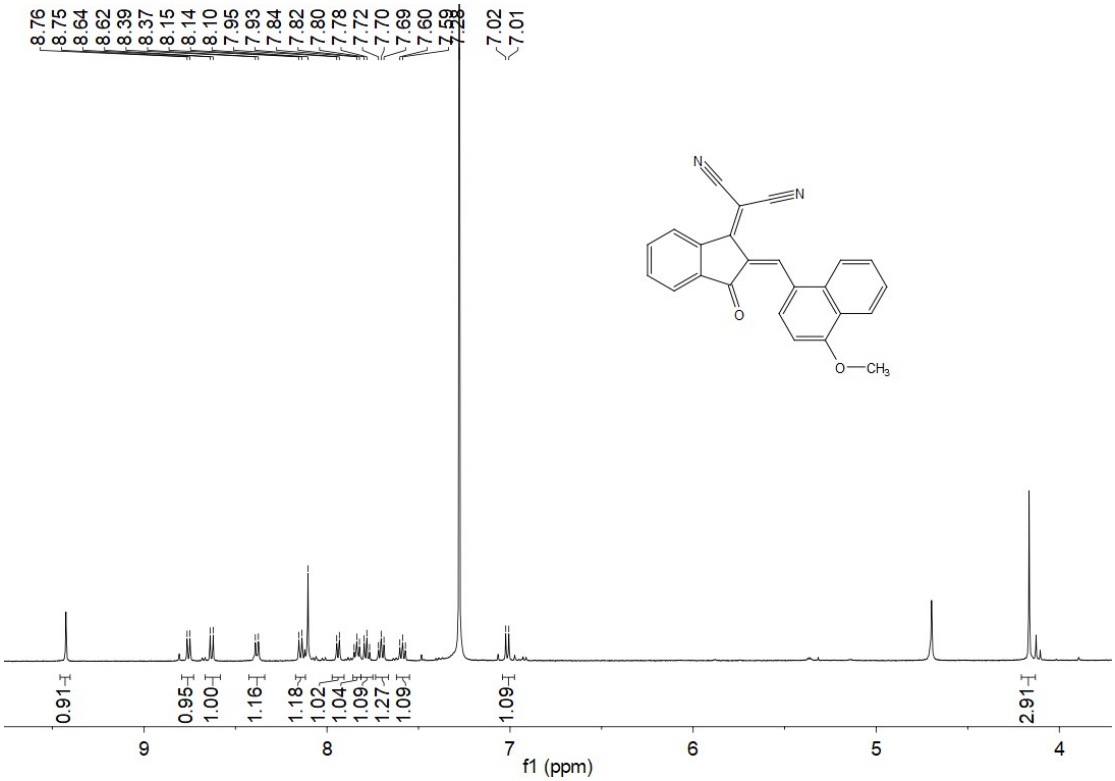


Figure S10. ^{1}H NMR spectrum of 4-MNIC in $CDCl_3$.

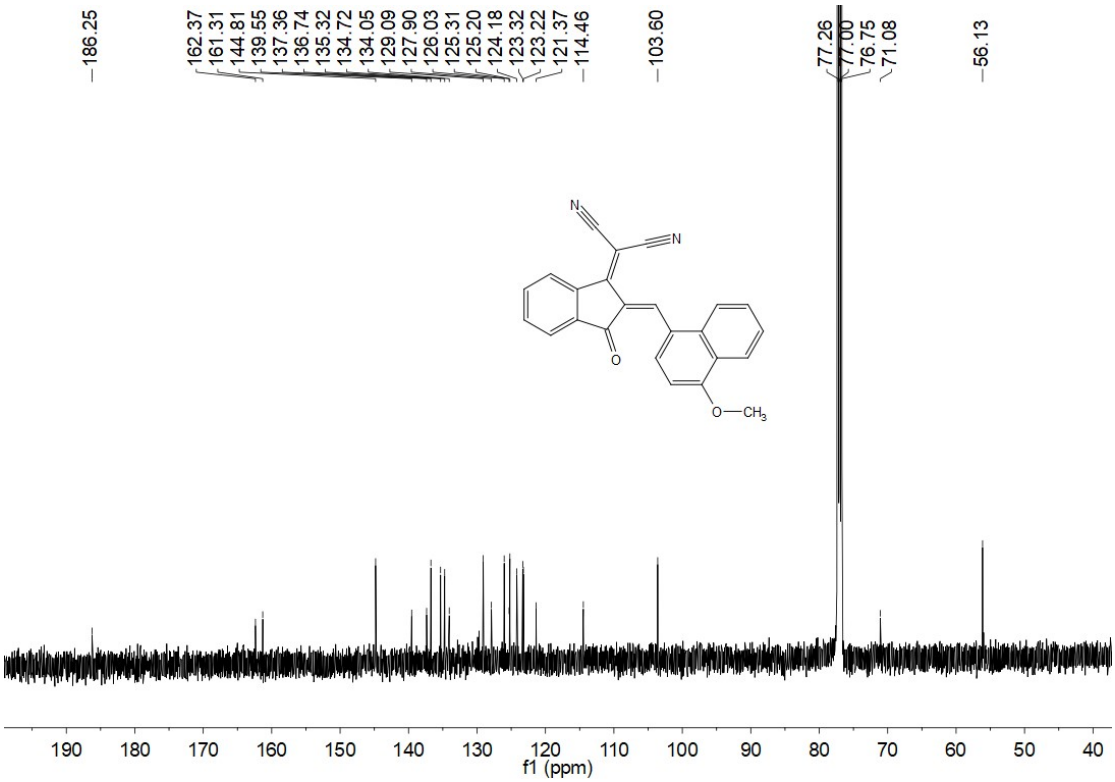


Figure S11. ^{13}C NMR spectrum of 4-MNIC in $CDCl_3$.

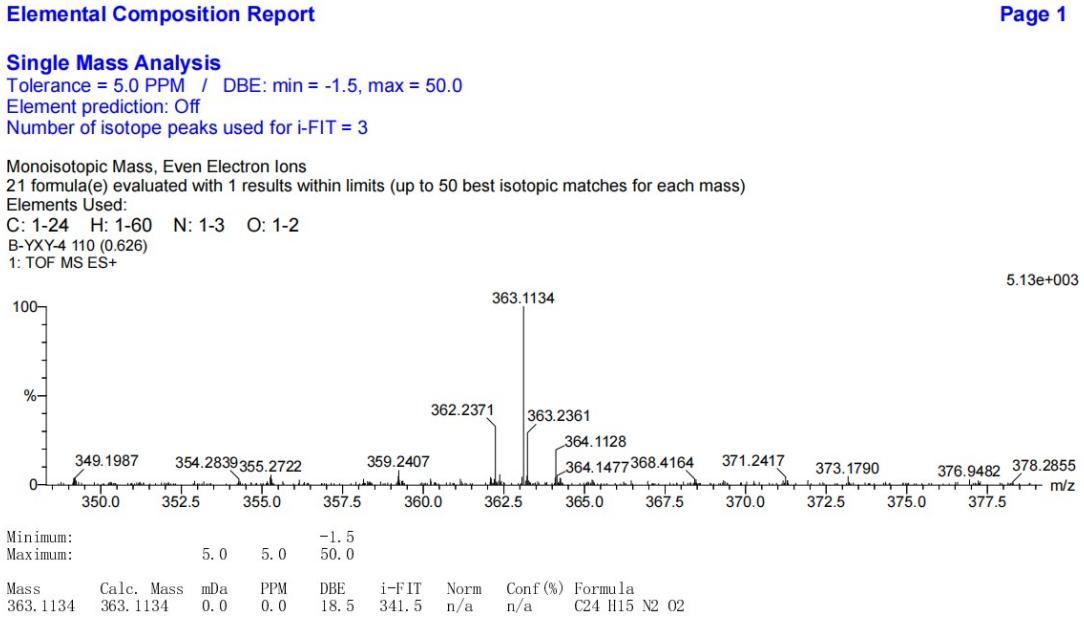


Figure S12. HRMS spectrum of 4-MNIC.

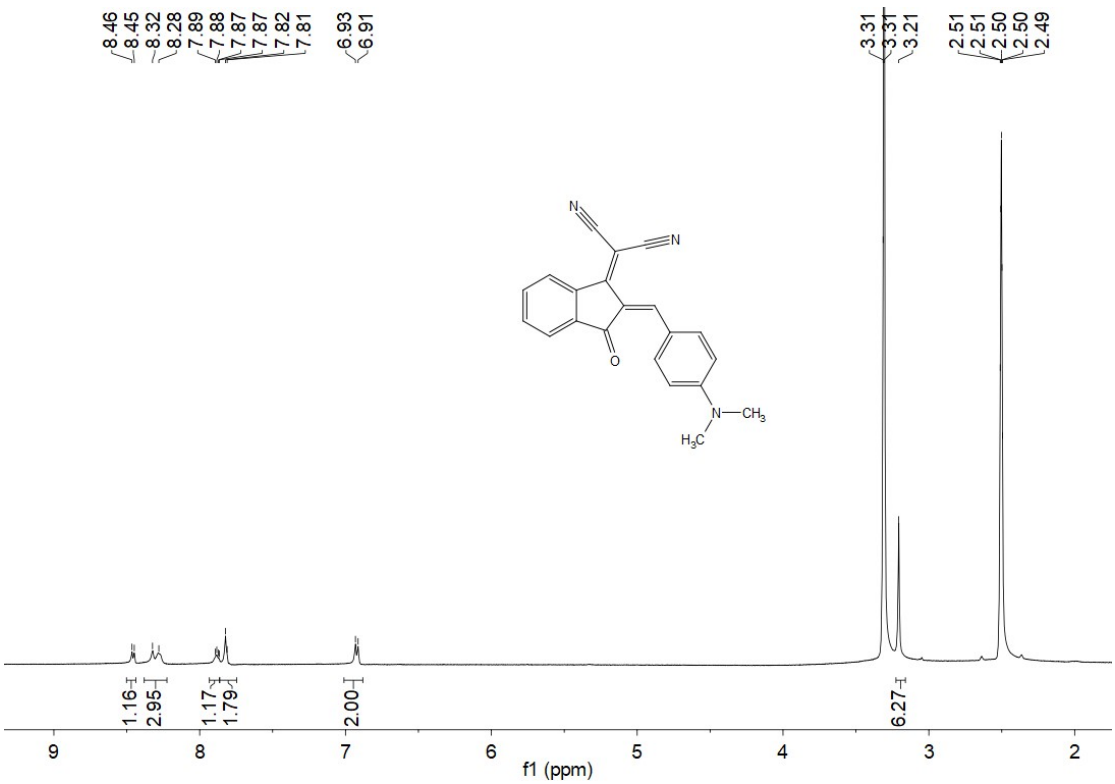


Figure S13. ^{1}H NMR spectrum of DMPIC in DMSO-d_6.

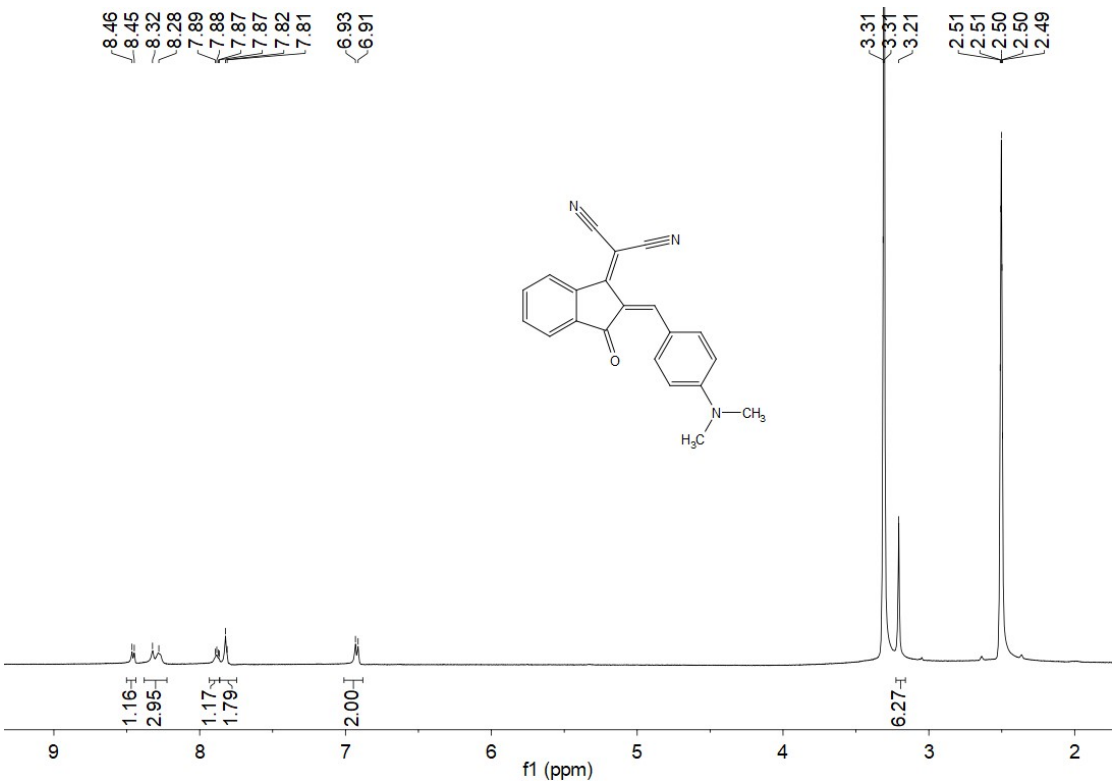


Figure S14. ^{13}C NMR spectrum of DMPIC in $CDCl_3$.

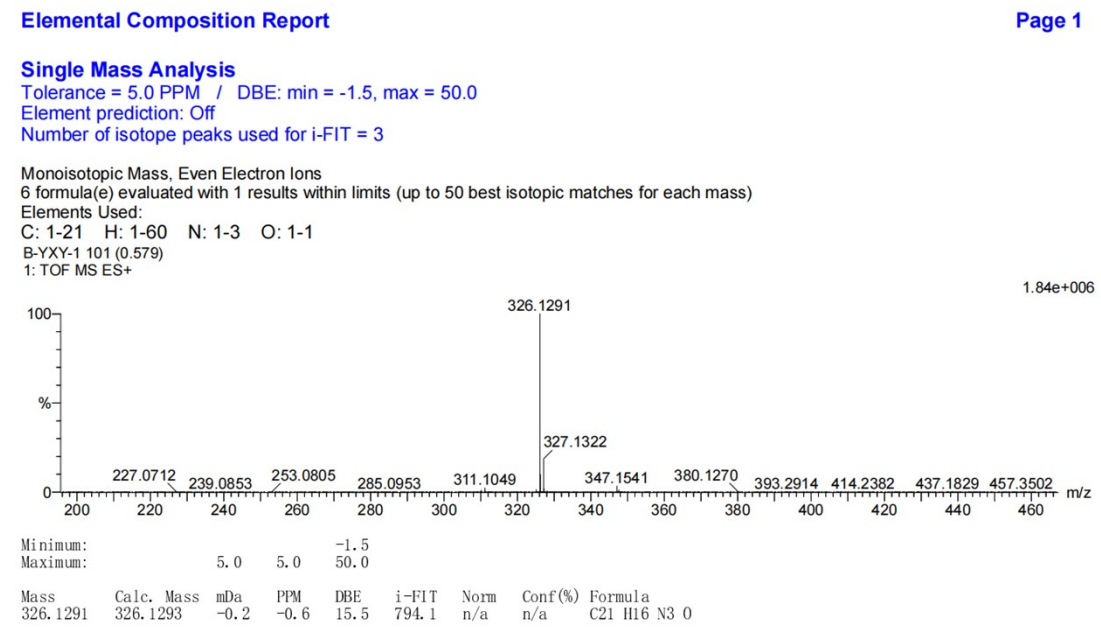


Figure S15. HRMS spectrum of DMPIC.

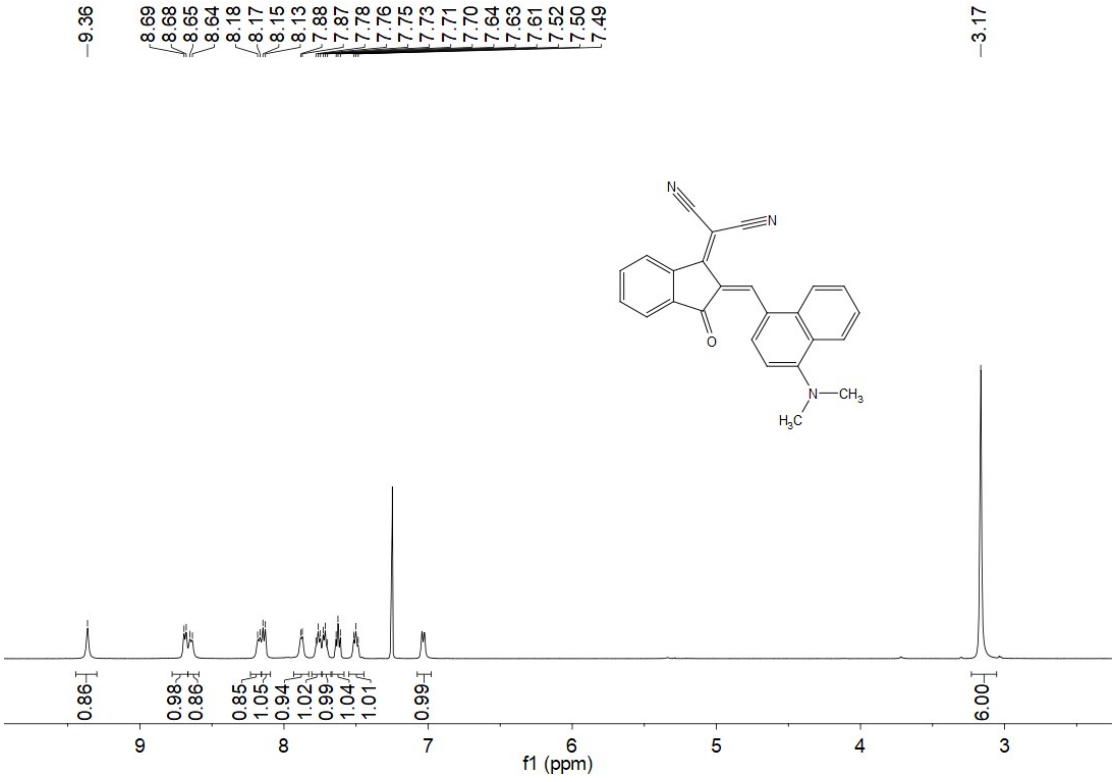


Figure S16. ^{1}H NMR spectrum of DMNIC in $CDCl_3$.

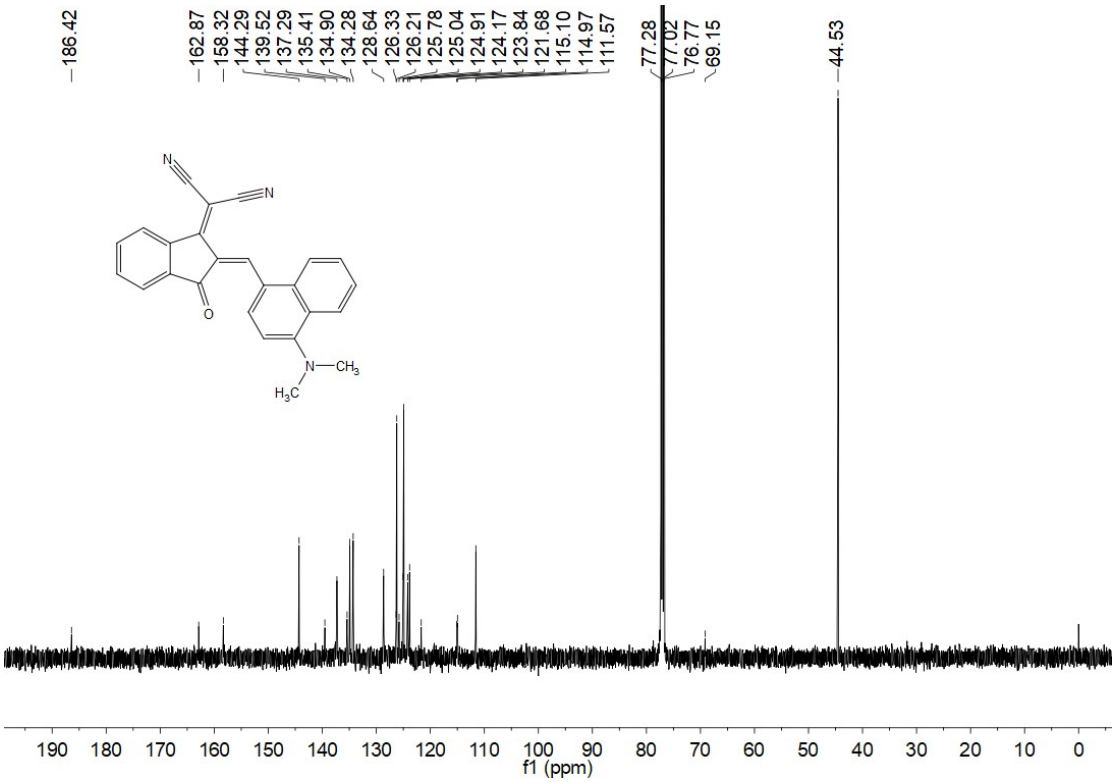


Figure S17. ^{13}C NMR spectrum of DMNIC in $CDCl_3$.

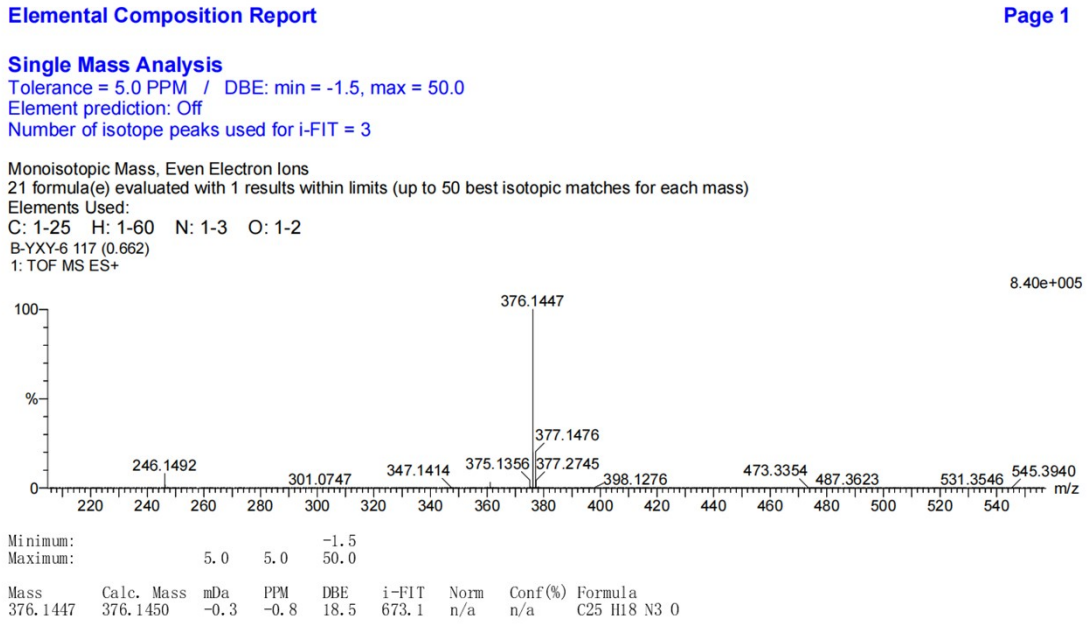


Figure S18. HRMS spectrum of DMNIC.

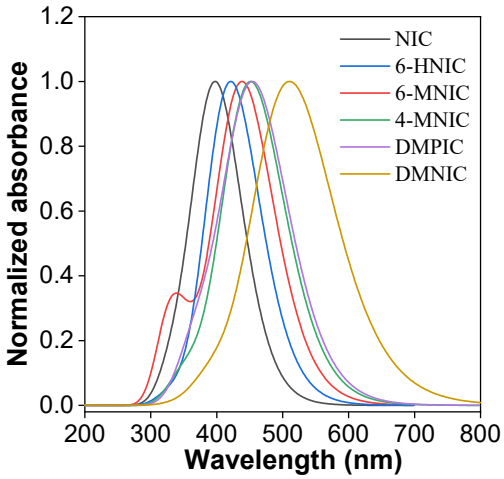


Figure S19. Absorption maxima of the six AIEgens calculated using TD CAM-B3LYP/6-311g(d) method.

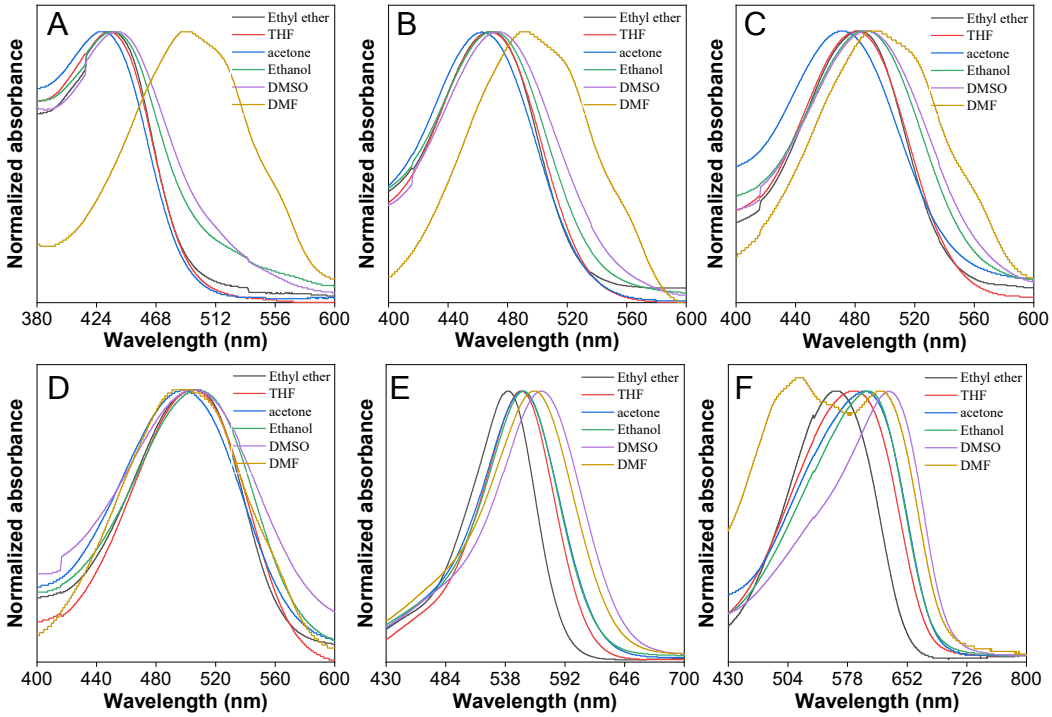


Figure S20. UV-vis absorption spectra of (A) NIC, (B) 6-MNIC, (C) 6-HNIC, (D) 4-MNIC, (E) DMPIC and (F) DMNIC in different solvents.

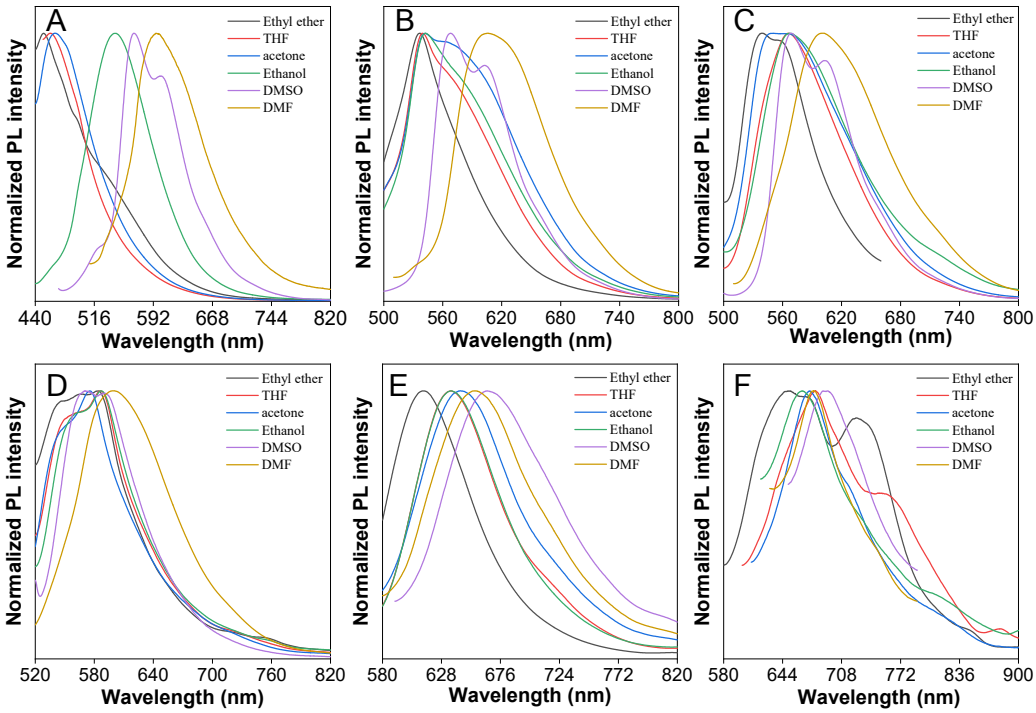


Figure S21. PL spectra of (A) NIC, (B) 6-MNIC, (C) 6-HNIC, (D) 4-MNIC, (E) DMPIC and (F) DMNIC in different solvents.

Table S1. Fluorescent quantum yields of six AIEgens and their nanoparticles.

Φ (%)[a]	NIC	6-MNIC	6-HNIC	4-MNIC	DMPIC	DMNIC
solution	0.3	0.2	0.2	0.2	0.2	0.2
aggregates	0.5	0.3	0.3	0.3	0.4	0.3
solid	0.9	8	0.7	1	0.9	0.6

[a] Φ = fluorescence quantum yield measured by using an integrating sphere.

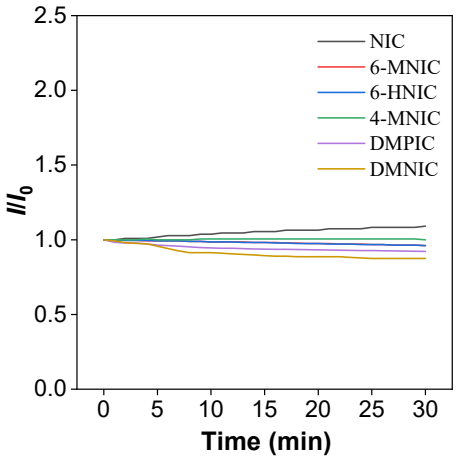


Figure S22. Photostability of the six AIEgens using a 50 W halogen lamp as the light source.

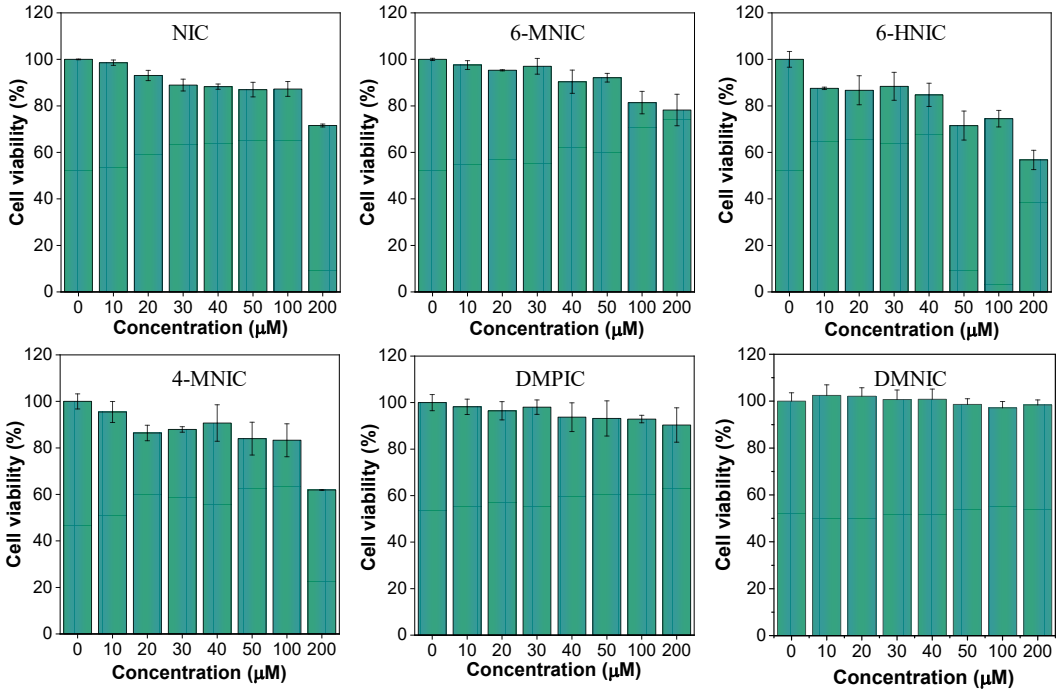


Figure S23. Cytotoxicity of the six AIEgens in the dark by MTT assay.

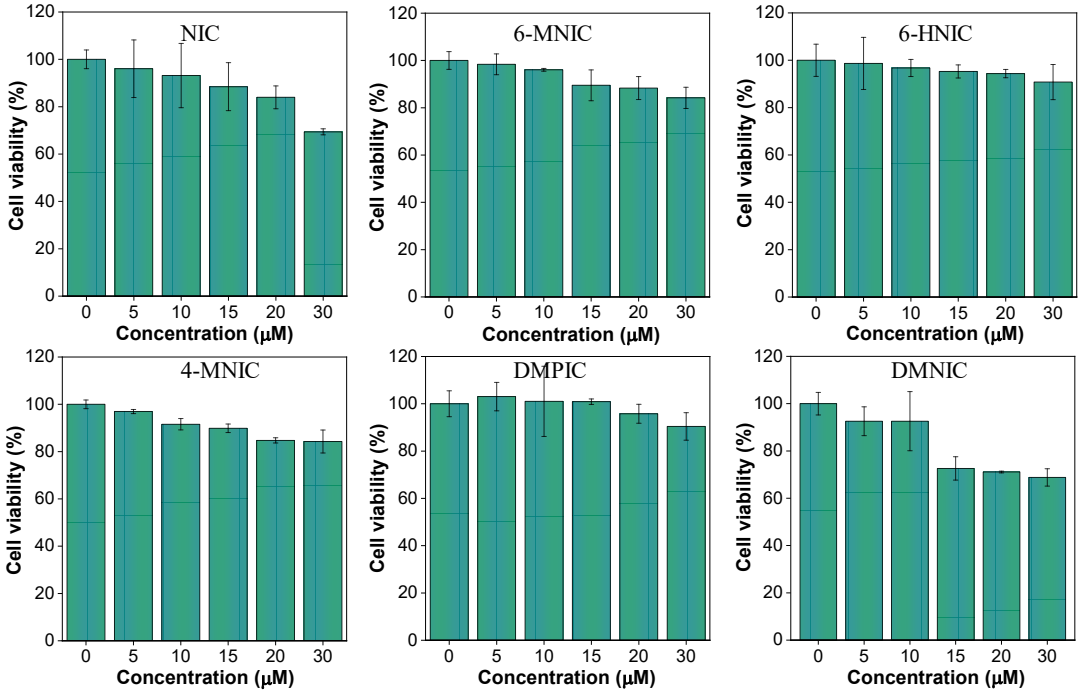


Figure S24. Cytotoxicity of the six AIEgens with light irradiation by MTT assay.