Supporting Information

Experimental section

1. Materials and apparatus

The reagents used in the chemical synthesis process are all analytically pure (AR) and further purified according to standard methods and the solvents used in the optical analysis and testing process are all chromatographically pure grade. Mass spectra were obtained on a Micromass GCT-MS Spectrometer (UK). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 spectrometer (Germany). UV-vis absorption spectra were recorded on a SHIMADZU UV-3600 spectrophotometer (Japan). Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer (Japan). Quantum yield was determined by FLUORMAX-4P /FLUORMAX-4P (Japan). Confocal microscopy imaging was acquired with a Leica SP8 (Germany) confocal microscopy and 100/63 × oil-immersion objective lens. The concentration of the mother liquor was 1.0×10^{-5} mol/L.

2. Synthetic procedures

The synthesis of N1: In a dry flask, indole-3 formaldehyde (2.9 g, 0.02 mol) was added to THF (50 mL) followed by slow addition of NaH (1.60 g, 0.06 mol) at 0 °C. After being stirred for 30 min, n-Ethyl bromide (2.62 g, 0.024 mol) was added to the reaction mixture. Then the reaction mixture was heated to reflux for 12 h. After completion of the reaction. The mixture was dissolved in CH_2Cl_2, the obtained organic layer was separated and washed with H_2O (20 mL) and then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting product was purified by silica gel column chromatography (petroleum ether: Ethylacetate = 5:1) to obtain grey crystal. Yield, 63%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.99 – 9.93 (m, 1 H), 8.34 – 8.25 (m, 1 H), 7.67 (d, *J* = 22.8 Hz, 1 H), 7.40 – 7.23 (m, 3 H), 4.30 – 4.09 (m, 2 H), 1.52 (t, *J* = 7.33 Hz, H). MS (APCI-MS): cal.:173.08, found: 174.0918 $[M+H]^{+}$.

The synthesis of N2: The synthesis step refers to **N1**. Yield, 64%. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1 H), 8.33 – 8.26 (m, 1 H), 7.66 (s, 1 H), 7.39 – 7.22 (m, 3 H), 4.12 (t, *J* = 7.22 Hz, H), 1.93 – 1.79 (m, 1 H), 1.34 – 1.23 (m, 2 H), 0.87 (t, *J* = 6.9 Hz, 3 H). MS (ESI-MS): cal.: 229.15, found: 230.1536 $[M+H]^{+}$.

The synthesis of N-2C: To a solution of **N1** (0.86 g, 0.005 mol) in ethanol (60 mL) was added 2-acetylpyridine (1.22 g, 0.011 mol), KOH (1.17 g, 0.020 mol), and aqueous ammonia (60 mL, 30%). The

reaction mixture was kept at 80 ℃ for 6 h. The precipitate formed after cooling and was collected by filtration. A yellow crystalline solid **N-2C** was obtained from recrystallization using ethanol. Yield, 69%. ^{1}H-NMR (400 MHz, *d6*-DMSO) δ 9.17 (s, 2 H,), 9.07 (d, *J* = 8.22 Hz, 2H,), 8.95 (m, 2 H), 8.46 (d, *J* = 9.12 Hz, 2H,), 8.34 (t, *J* = 9.11 Hz, 1H,), 7.98 (d, *J* = 5.2, 2 H,), 7.51 – 7.46 (m, 2 H), 7.43 – 7.38 (m, 1 H), 7.33 (d, *J* = 6.21Hz, 1H), 4.47 – 4.30 (m, 2 H), 1.48 – 1.51 (m, 3 H). MS (APCI-MS): cal.: 376.17, found: 377.0963 $[M+H]^{+}$.

The synthesis of N-6C: The synthesis step refers to **N-2C**. Yield, 58%. ^{1}H-NMR (400 MHz, *d6*-DMSO) δ 9.15 (s, 2 H,), 9.03 (d, *J* = 8.10 Hz, 2H,), 8.91 (s, 2 H), 8.36 (d, *J* = 8.02 Hz, 2H,), 8.24 (t, *J* = 7.61 Hz, 1H,), 7.93 (d, *J* = 4.8, 2 H,), 7.52 – 7.45 (m, 2 H), 7.41 – 7.36 (m, 1 H), 7.32 (d, *J* = 3.61Hz, 1H), 4.45 – 4.20 (m, 2 H), 1.96 – 1.82 (m, 2 H), 1.33 – 1.27 (m, 6 H), 0.82 (dd, *J* = 15.4 Hz, 7.0 Hz, 3 H). MS (ESI-MS): cal.: 432.23, found: 433.2379 $[M+H]^{+}$.

The synthesis of Zn-2C: Dissolve the ligand **N-2C** (0.37 g, 1 mmol) in methanol, then dissolve zinc nitrate (0.18 g, 0.7 mmol) in methanol and gradually add dropwise to the above solution, reflux at 80 °C for 3 hours, stop the reaction, cool, and filter with suction, recrystallized from methanol to obtain a yellow solid 1.01 g. Yield: 68%. ^{1}H-NMR (400 MHz, *d6*-DMSO) δ 9.14 (s, 3 H), 9.01 (d, *J* = 7.91 Hz, 3H), 8.92 (s, 4H), 8.78 (s, 2H), 8.38 (d, *J* = 8.41 Hz,4 H), 8.25 (d, *J* = 7.31 Hz, 2H,), 7.91 (s, 4 H), 7.72 (d, *J* = 7.00 Hz, 2H,), 7.45 (d, *J* = 8.11 Hz, 2H,), 7.35 (d, *J* = 17.84, 4H), 4.38 (dd, *J* = 17.9 Hz, 8.9 Hz, 2 H), 1.57 – 1.45 (m, 6 H). ^{13}C-NMR (400 MHz, *d6*-DMSO) δ 15.6, 43.8, 102.5, 114.8, 118.5, 119.7, 122.6, 123.3, 124.7, 128.1, 130.3, 130.4, 137.1, 141.4, 148.1, 149.7, 152.9, 154.6. MS (ESI-MS): cal.: 960.24, found: 408.25 $[M-2NO_3^-]^{2+}/2$.

The synthesis of Zn-6C: The synthesis method was referred to **Zn-2C**, recrystallized from methanol to obtain a yellow solid 1.12 g. Yield: 63%. ^{1}H-NMR (400 MHz, *d6*-DMSO) δ 9.19 (s, 2H), 9.07 (d, *J* = 8.1 Hz, 2H), 9.01 – 8.82 (m, 6H), 8.69 (s, 2H), 8.48 (d, *J* = 34.9 Hz, 4H), 8.29 (t, *J* = 7.7 Hz, 2H), 7.97 (d, *J* = 4.6 Hz, 4H), 7.84 – 7.69 (m, 2H), 7.58 – 7.47 (m, 2H), 7.44 – 7.34 (m, 4H), 4.38 (d, *J* = 32.5 Hz, 4H), 1.96 (s, 4H), 1.48 – 1.18 (m, 12H), 0.88 (t, *J* = 7.6 Hz, 6H). ^{13}C-NMR (400 MHz, *d6*-DMSO) δ 14.0, 22.9, 27.8, 29.9, 32.1, 57.1, 100.4, 109.8, 118.3, 120.0, 121.6, 122.5, 122.7, 125.1, 128.3, 130.4, 135.6, 139.4, 148.1, 153.0, 156.7, 157.2. MS (ESI-MS): cal.: 1052.37, found: 464.42 $[M-2NO_3^-]^{2+}/2$.

3. Live cell experiment

Cell Culture. Cells were cultured in 25 cm^2 culture flasks in DMEM, supplemented with fetal bovine serum (10 %), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37°C in a CO_2 incubator (95 %

relative humidity, 5 % CO_2). Cells were seeded in 35 mm glass bottom cell culture dishes, at a density of 1 × 10^5 cells and were allowed to grow when the cells reached more than 60 % confluence. The two complexes were dissolved in DMSO with concentration of 1mM as stock solution, and the commercial dyes were prepared as 1mM PBS solution and diluted to working concentration as protocol required.

Cytotoxicity assay. To ascertain the cytotoxic effect of all the compounds' treatment over a 24 h period, luminescent cell viability assay was performed by Cell Titer-Lumi™ Plus Luminescent Cell Viability Assay Kit (Beyotime, China). HeLa cells were trypsinized and plated to 70 % confluence in 96 well black plates 24 h before treatment. All complexes were then added at indicated concentrations to triplicate wells. Prior to the complexes' treatment, the DMEM was removed and replaced with fresh DMEM, and aliquots of the compounds stock solutions were diluted to obtain the final concentrations of 1, 2.5, 5 and 10 μM. The treated cells were incubated for 24 hours at 37 °C and under 5 % CO_2. Subsequently, the cells were treated with mixture of detection reagent and DMEM (ratio is 1:1) for 10 min (37 °C, 5 % CO_2). Then, the chemiluminescence value of each well can be measured by using a microplate reader (SpectraMax Paradigm).

Intracelluar ROS detection. Cells in exponential growth phase were digested with trypsin and seeded on 35 mm glass-bottomed cell culture dishes at a density of 1 × 10^5 cells. When the cells reached about 70% confluence, followed by incubation with Zn-nC (1 μM) for 10 min at 37°C in 5 % CO_2, and then washed with PBS for three times. Then 1mL of DCFH-DA solution with concentration of 10 μM was added. After lighting for 20 min, the groups of cells were imaged by a confocal microscope.

4. Immunofluorescence

Cells in exponential growth phase were digested with trypsin and seeded on 35 mm glass-bottomed cell culture dishes at a density of 1 × 10^5 cells. When the cells reached about 80% confluence, followed by incubation with Zn-nC (1 μM) for 10 min, then were treated under ROS induced conditions (405 nm lasers, 5 min, interval = 30 s). Prefixed cell was applied 0.5 % Triton X-100 for 5 min and washed by PBS 3 times for 5 min every time. After incubated with 100 mM glycine for 15 min at room temperature, PBS washed the cell again for 5 min every time. Posteriorly, the cell was covered with 1% BSA for 1 hour in order to close the non-specific binding sites for primary antibody and incubated by using primary antibodies in the 4degree refrigerator for more than 12 hours. After washing by PBS 3 times for 10 min every time,

incubated with fluorescent second antibodies for 1 hour without exposure of light. The imaging was carried out after the cell washed by PBS for 3 times.

5. Annexin V-FITC/PI apoptotic assay

HeLa cells were seeded in a Petri dish 3.5 cm in diameter, incubated with Zn-nC (1 μM) for 2 h at 37°C in 5% CO_2 when the cells reached more than 80 % confluence. When the cells reached about 80% confluence, followed by incubation with Zn-nC (1 μM) for 10 min, then were treated under ROS induced conditions (405 nm lasers, 5 min, interval = 30 s). Next the cells were washed by PBS 3 times, and then used the Annexin V-FITC/PI Apoptosis Detection kit (BD Biosciences) to detect the apoptosis following the manufacturer's protocols .

6. Microscopy

Confocal imaging. Confocal microscopy imaging was acquired with a Leica TCS SP8 confocal microscopy and 40X objective lens, 63X/100X oil-immersion objective lens. The incubated slices were excited at 405 nm for onephoton imaging, 633 nm for NucRed, 405 nm for DAPI, 488 nm for Syto-9, 488/650 nm for fluorescent second antibodies with a semiconductor laser, and the emission signals were collected at 680 ± 20 nm for NucRed, 430 ± 20 nm for DAPI, 520 ± 20 nm for Syto-9, 520 ± 20/ 680 ± 20 nm for fluorescent second antibodies, respectively.

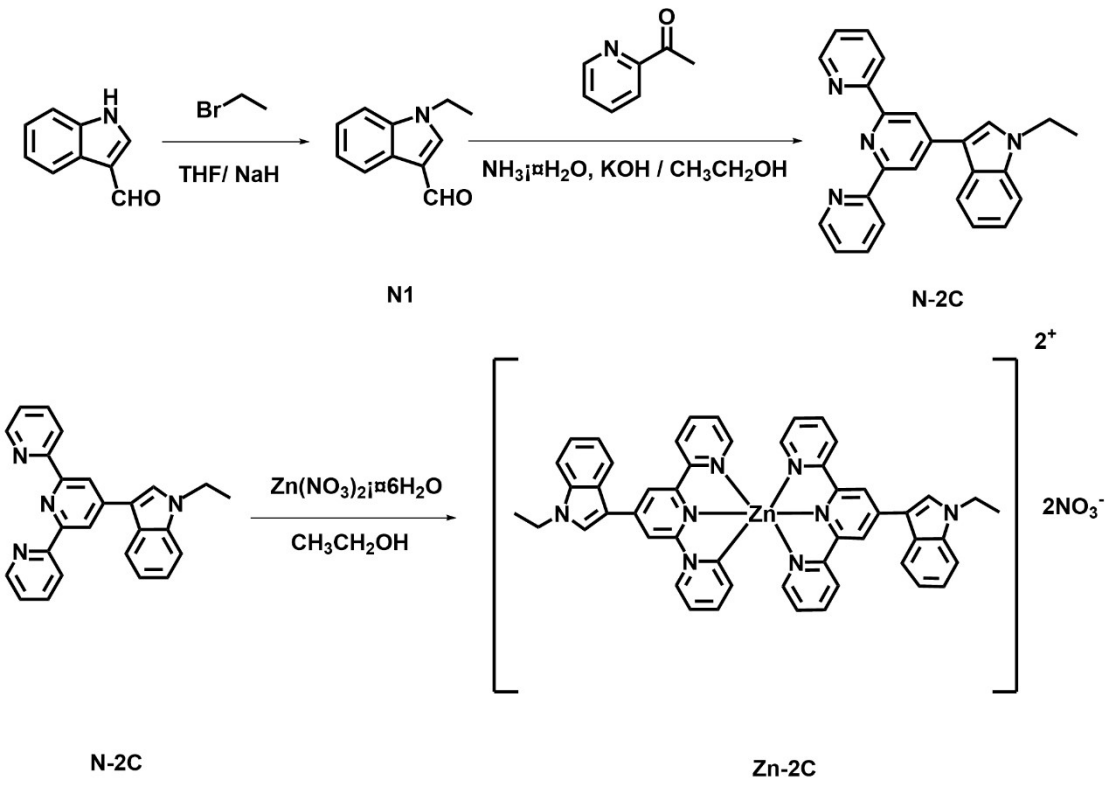
STED imaging. STED nanoscopy experiments was performed under Leica DMi8 confocal microscopy equipped with Leica TCS SP8 STED-ONE unit and the compound was excited under STED laser, the emission signals were collected using HyD reflected light detectors (RLDs). Specimen living cells were prepared using similar method as normal confocal microscopy described previously, and donut laser used in 660nm STED laser (70 % power), with 2048*2048 pixel and *100 scanning speed. The STED micrographs were further processed 'deconvolution wizard' function using Huygens Professional software (version: 16.05) under authorized license. The area radiuses were estimated under 0.02 micros with exclusion of 100 absolute background values.

7. Image processing and analysis

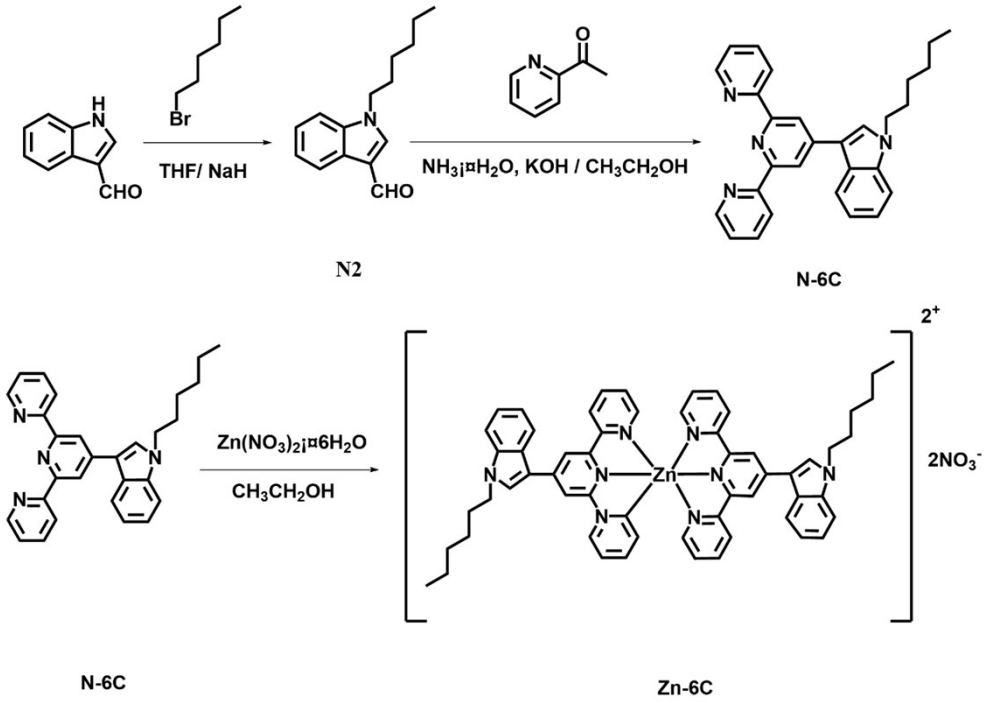
Micrographs were processing and analyzed by Huygens software and ImageJ 1.48 v (32-bit).

Quantification of the fluorescence intensity was achieved via Analyze >> Tools >> ROI manager in ImageJ from three parallel experiments. Quantification of single cell intensity profile was achieved via Analyze >> Plot Profile by selecting one cell in ImageJ. Quantification of colocolization coefficency was achieve via an external plugin via Plugins >> Colocolization Finder.

Supporting Schemes and Figures



Scheme S1 Synthetic procedures for target molecules Zn-2C



Scheme S2 Synthetic procedures for target molecules Zn-6C.

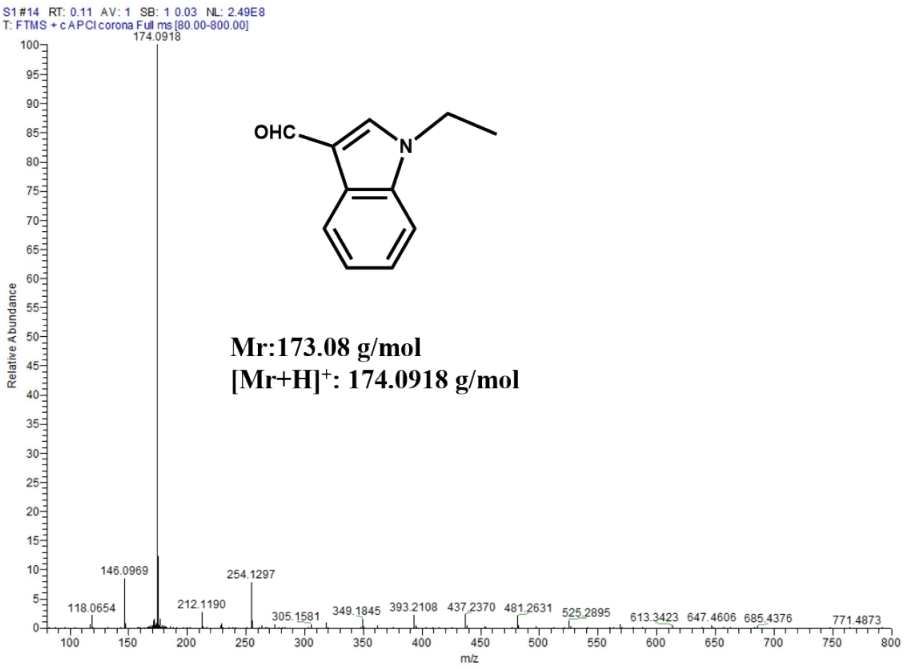


Figure S1 Mass spectra of **N1**.

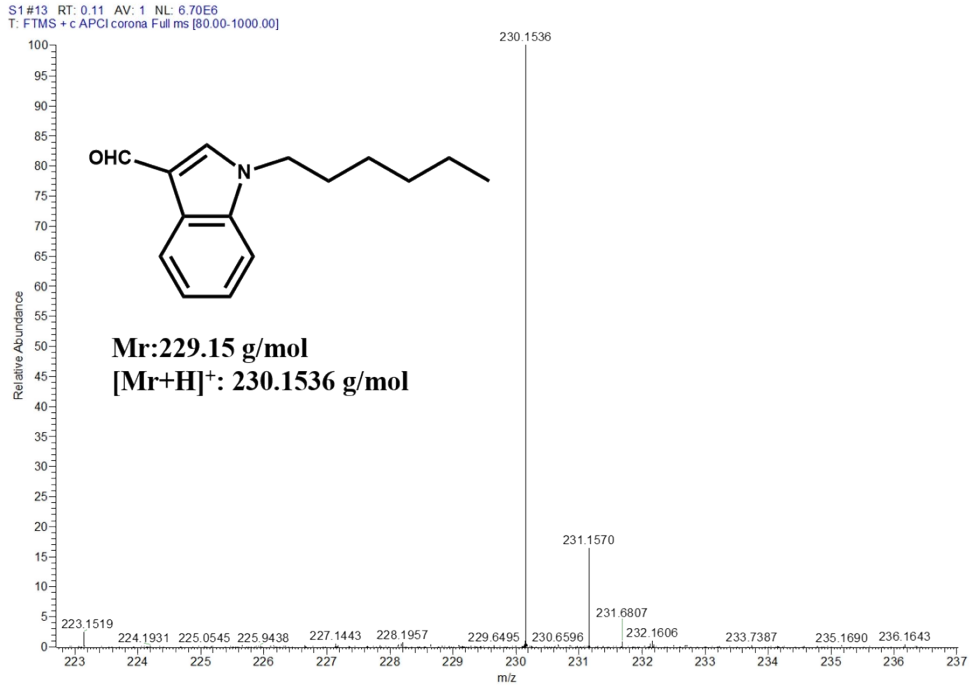


Figure S2 Mass spectra of **N2**.

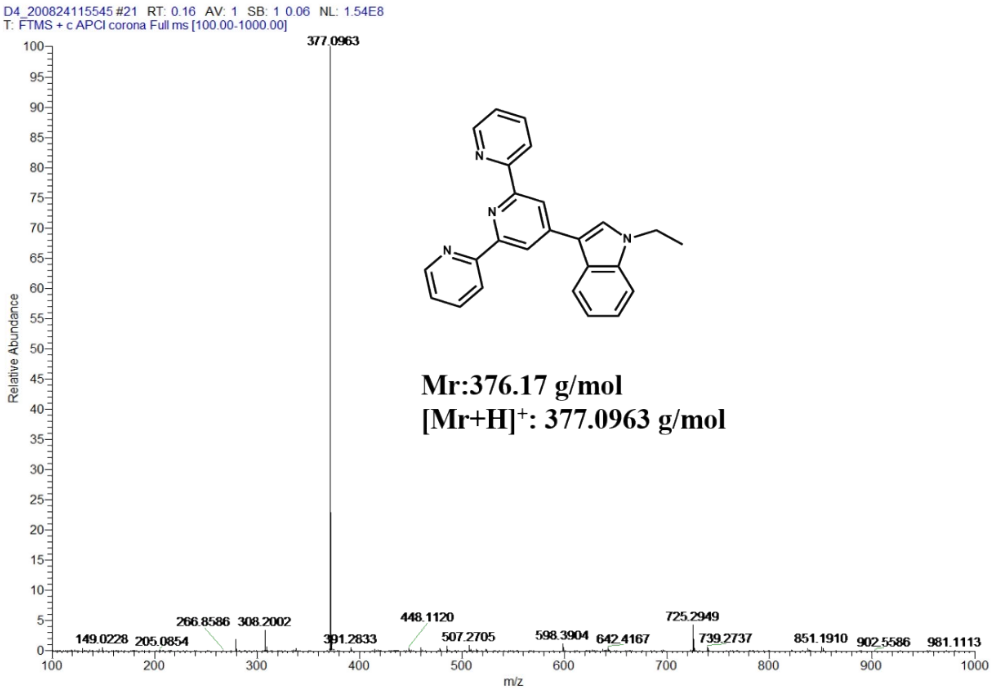


Figure S3 Mass spectra of **N-2C**.

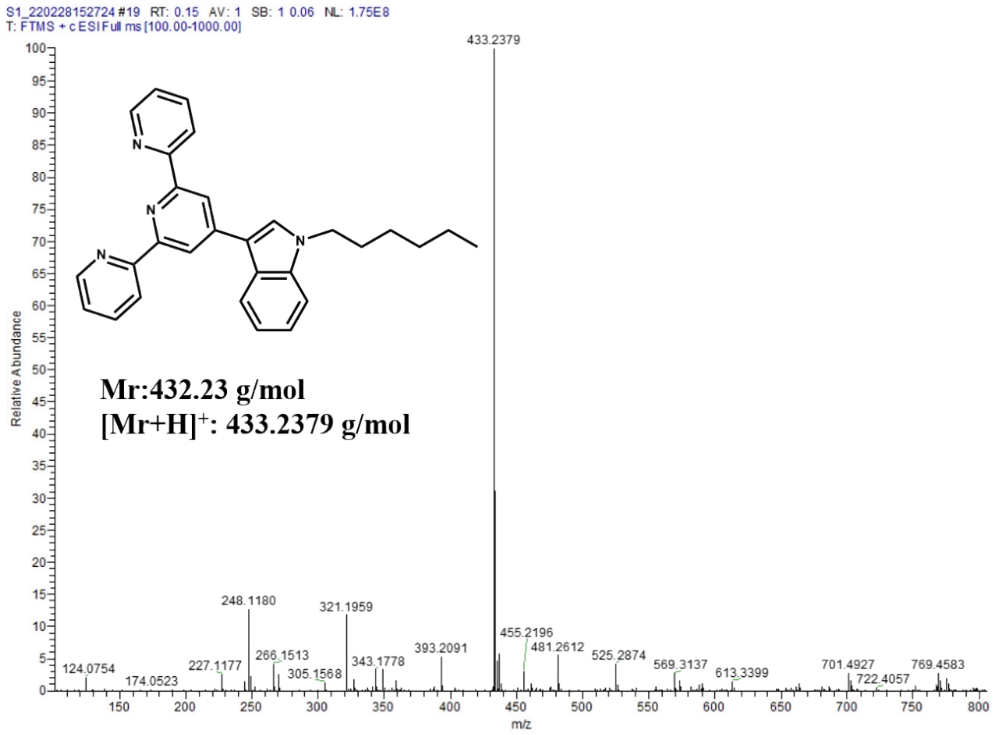


Figure S4 Mass spectra of **N-6C.**

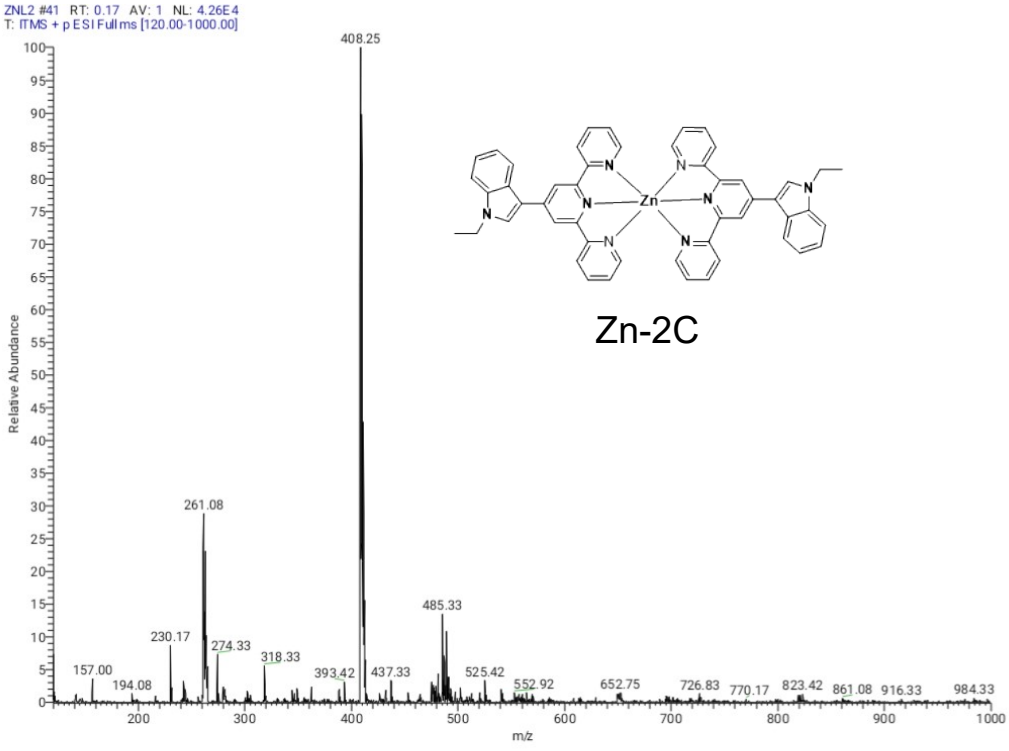


Figure S5 mass spectra of **Zn-2C**.

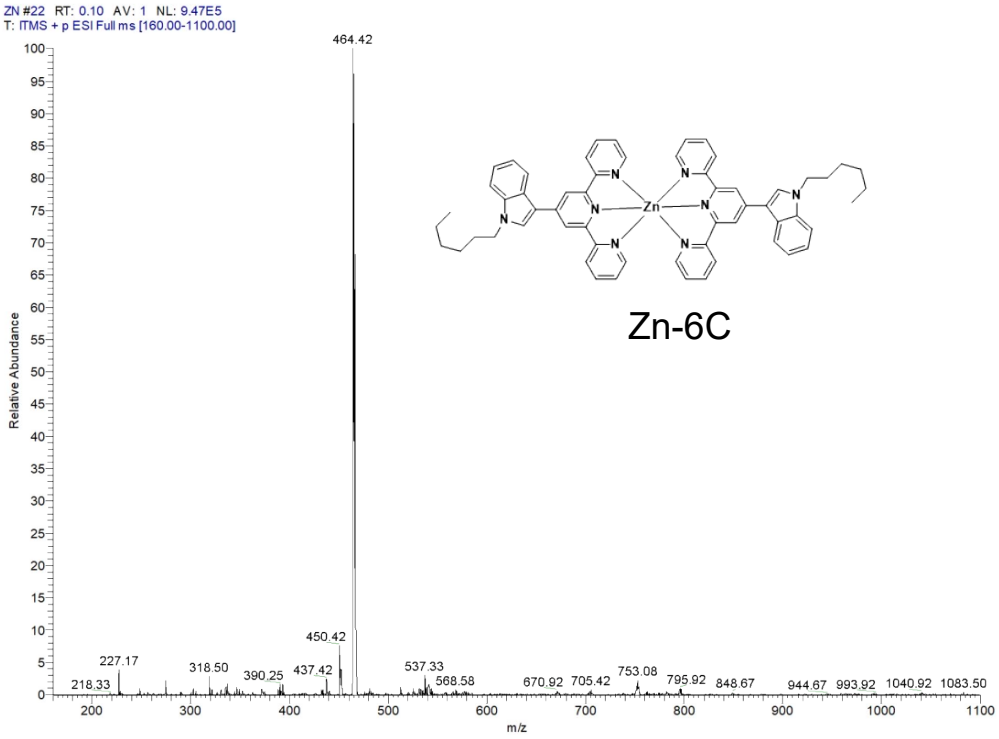


Figure S6 mass spectra of Zn-6C.

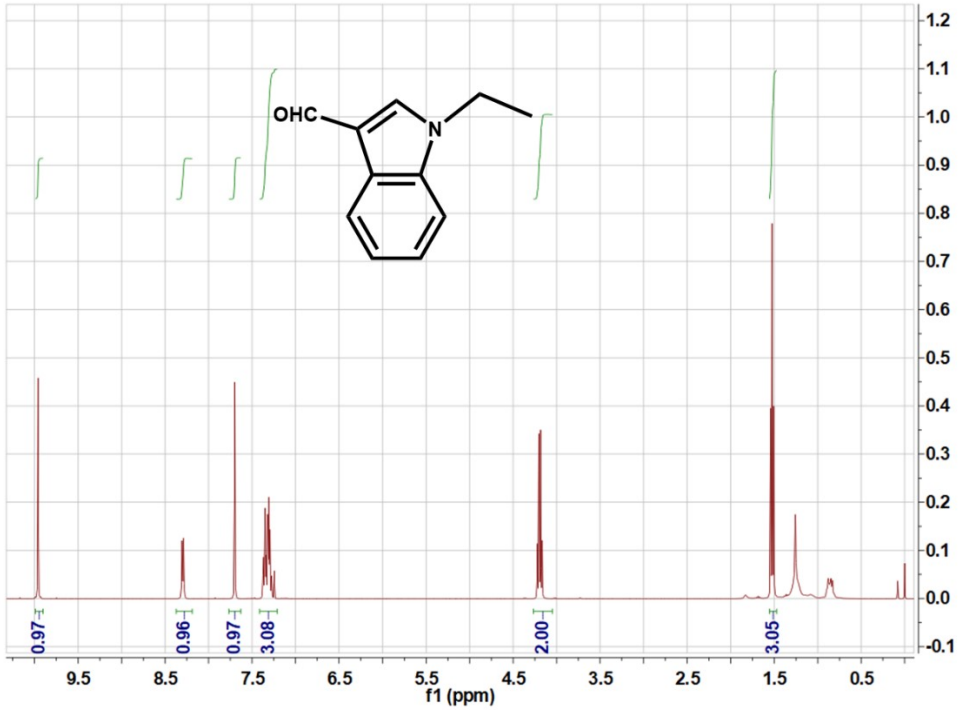


Figure S7 ^{1}H NMR spectrum of **N1** (CDCl3).

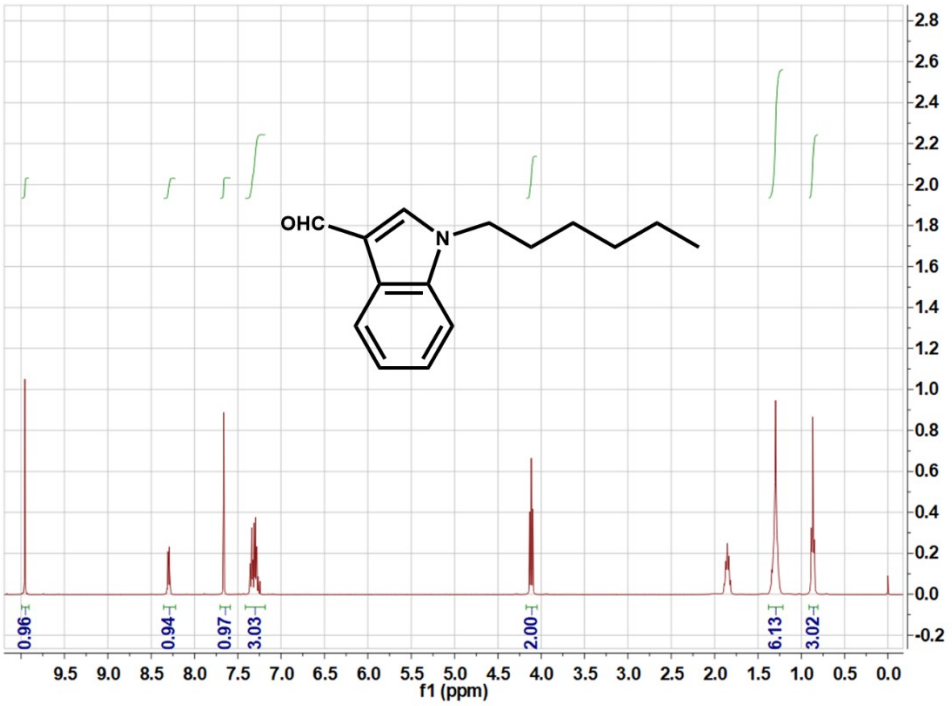


Figure S8 ^{1}H NMR spectrum of **N2** (CDCl3).

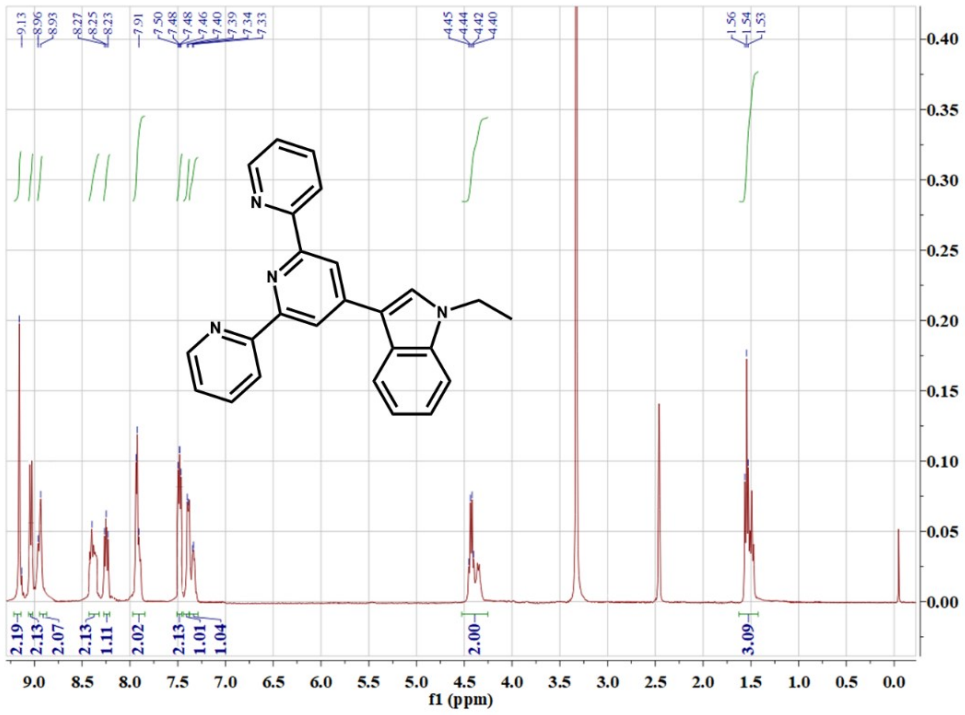


Figure S9 ^{1}H NMR spectrum of **N-2C** (d_6-DMSO).

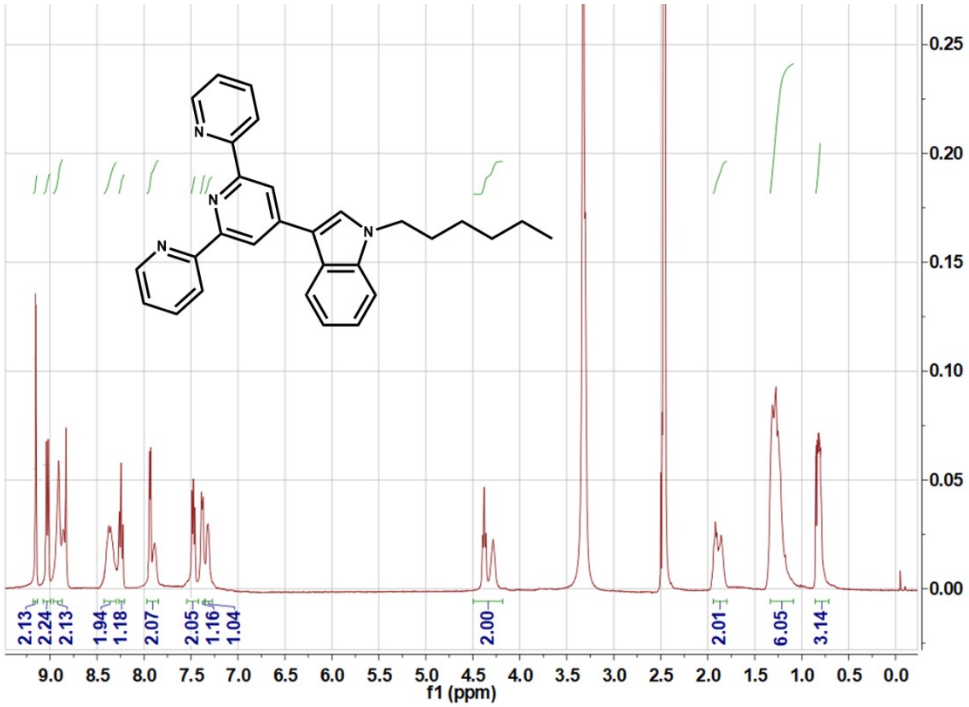


Figure S10 ^{1}H NMR spectrum of **N-6C** (d_6-DMSO).

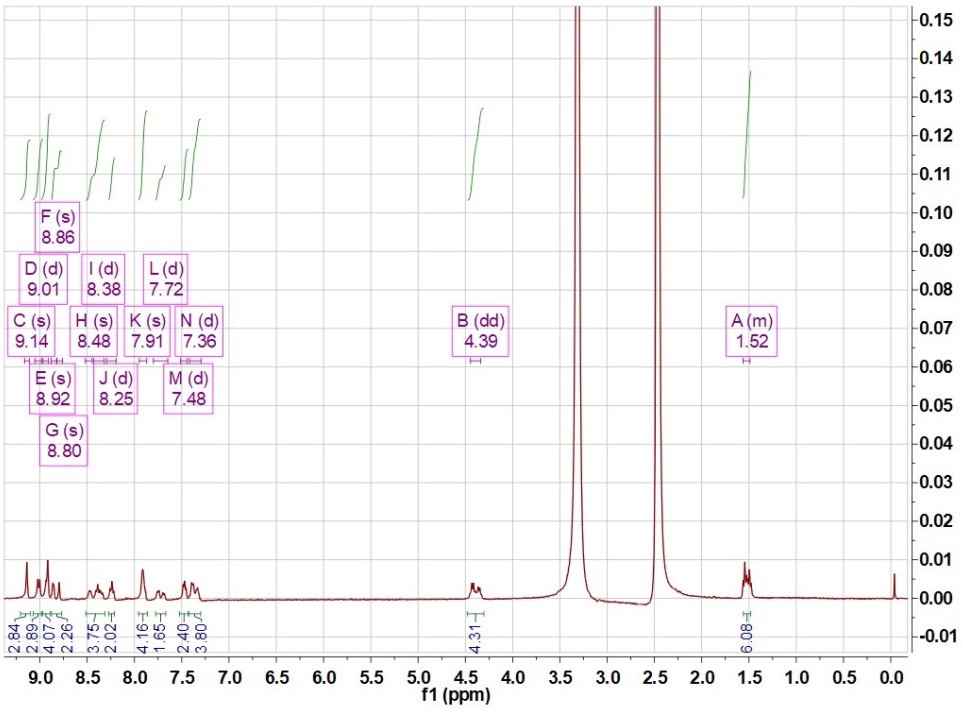


Figure S11 ^{1}H NMR spectrum of **Zn-2C** (d_6-DMSO).

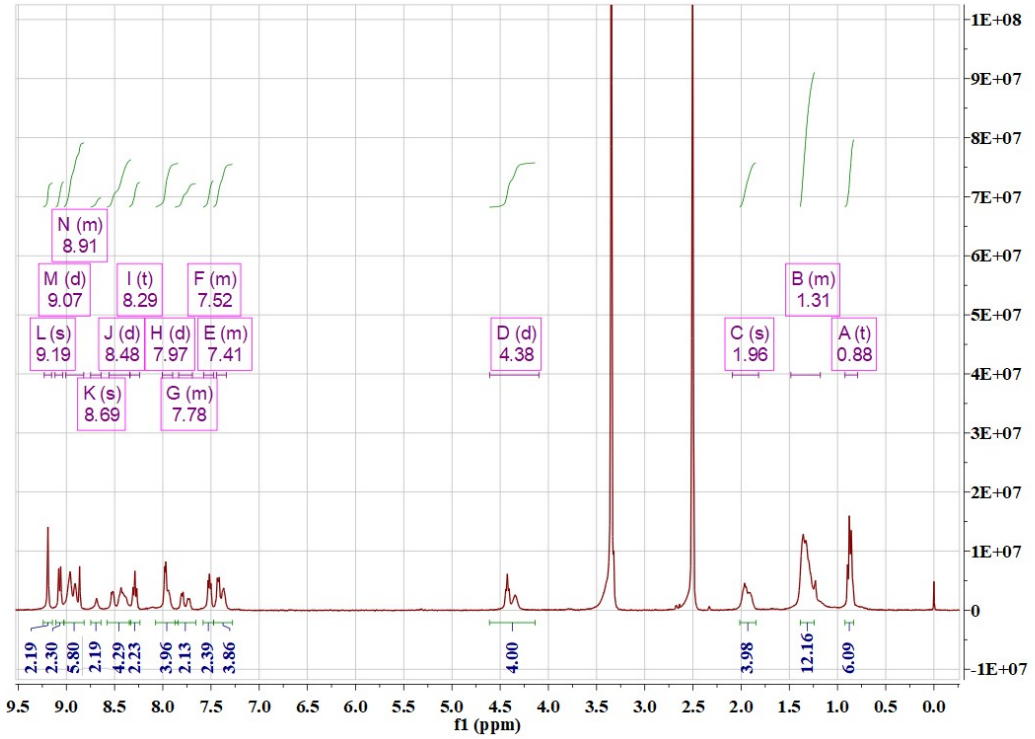


Figure S12 ^{1}H NMR spectrum of **Zn-6C** (d_6-DMSO).

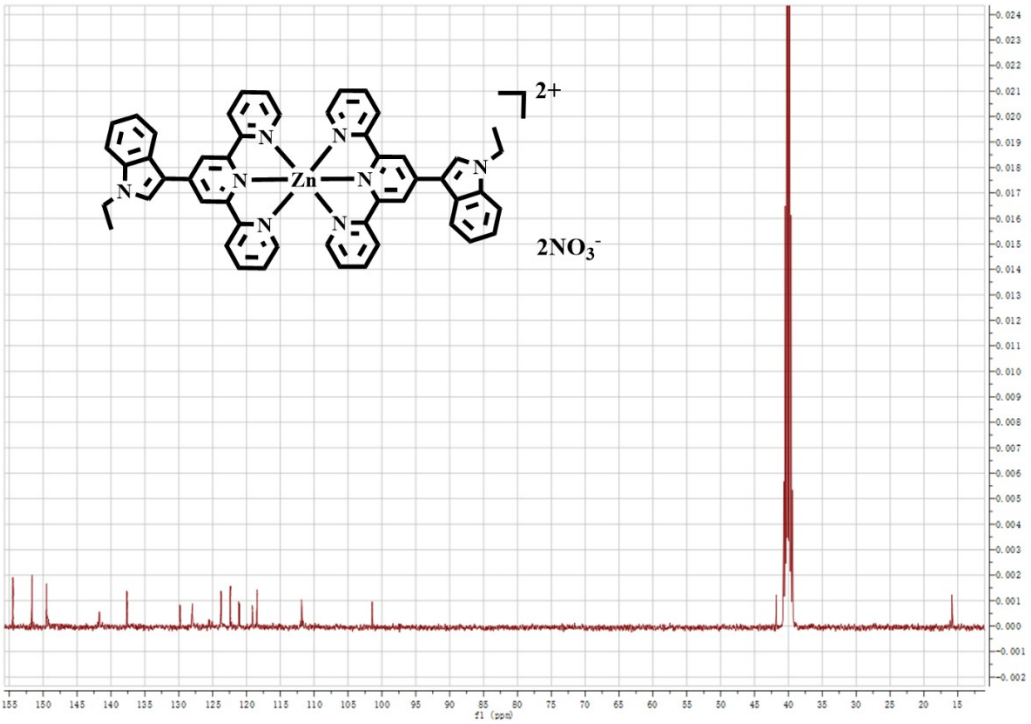


Figure S13 ^{13}C NMR spectrum of **Zn-2C** (d_6-DMSO).

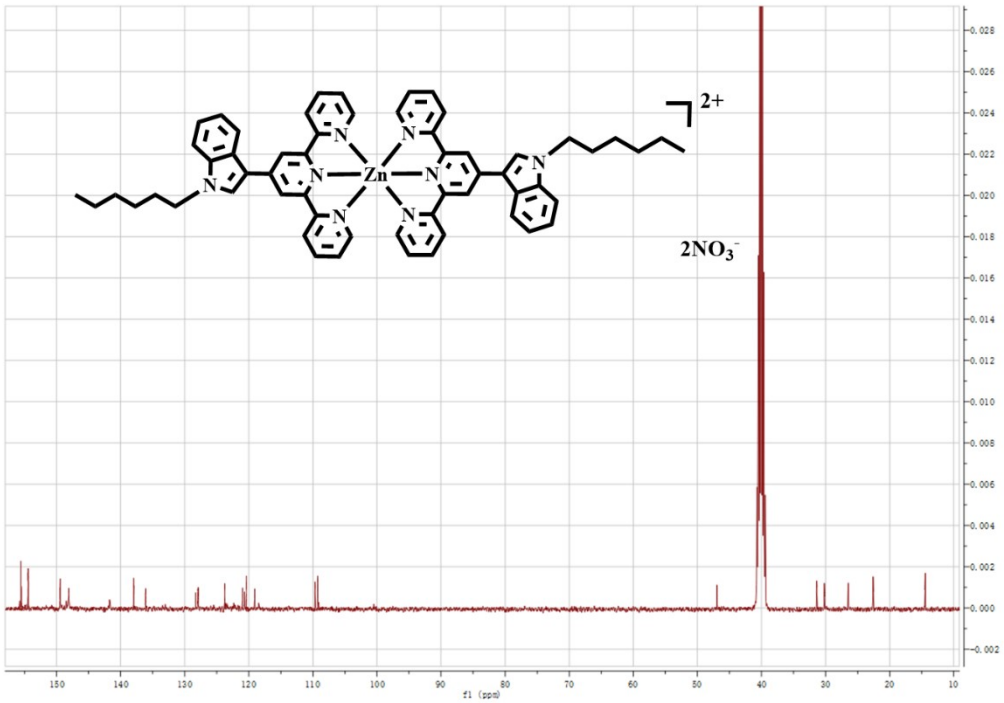


Figure S14 ^{13}C NMR spectrum of **Zn-6C** (d_6-DMSO).

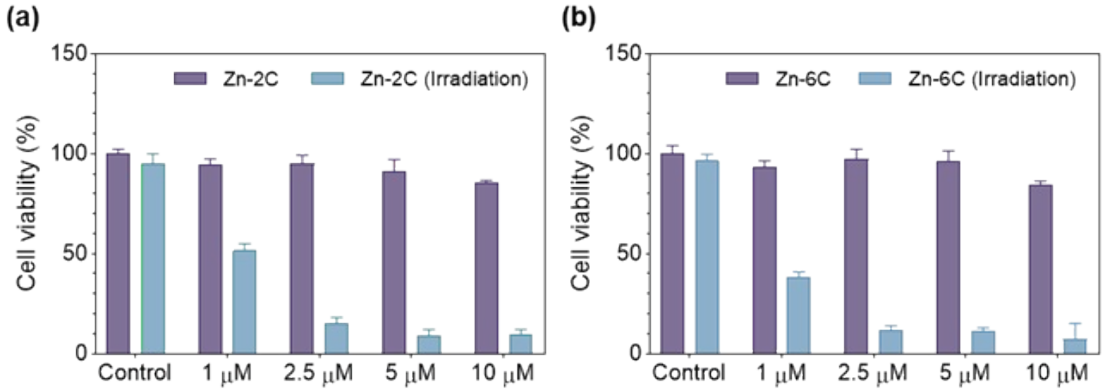


Figure S15 Cytotoxicity assay. Dark toxicity and phototoxicity of Zn-2C (a) and Zn-6C (b) under different concentration for 24 hours. (Irradiation time: 375 nm,0.2 mW/cm^2).

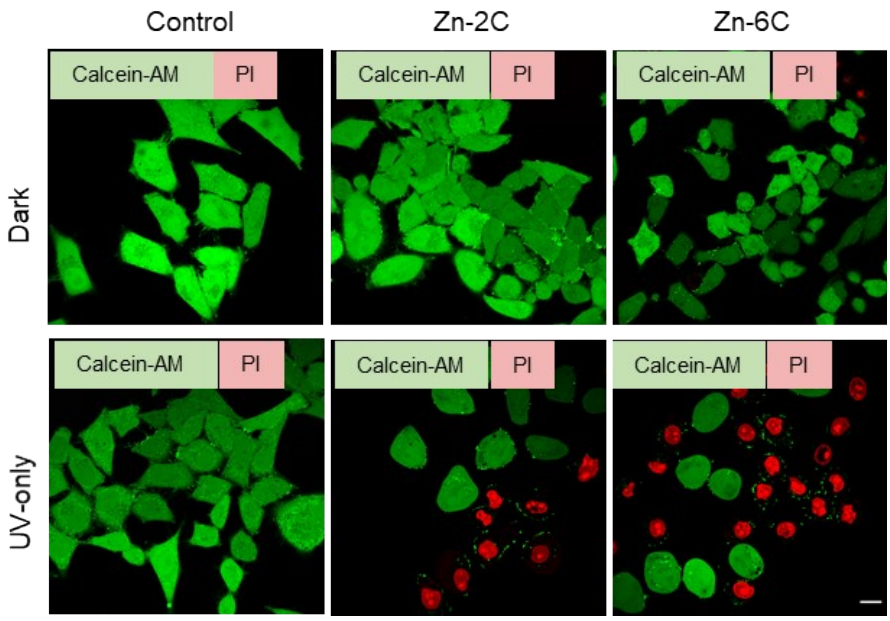


Figure S16 The live/dead cells were differentiated by calcein AM (live cells, green fluorescence) and PI (dead cells, red fluorescence) co-staining after Zn-nC (1 μM) treatment with or without ROS induced conditions (405 nm lasers, 5 min, interval = 30 s). Scale bar = 20 μm.

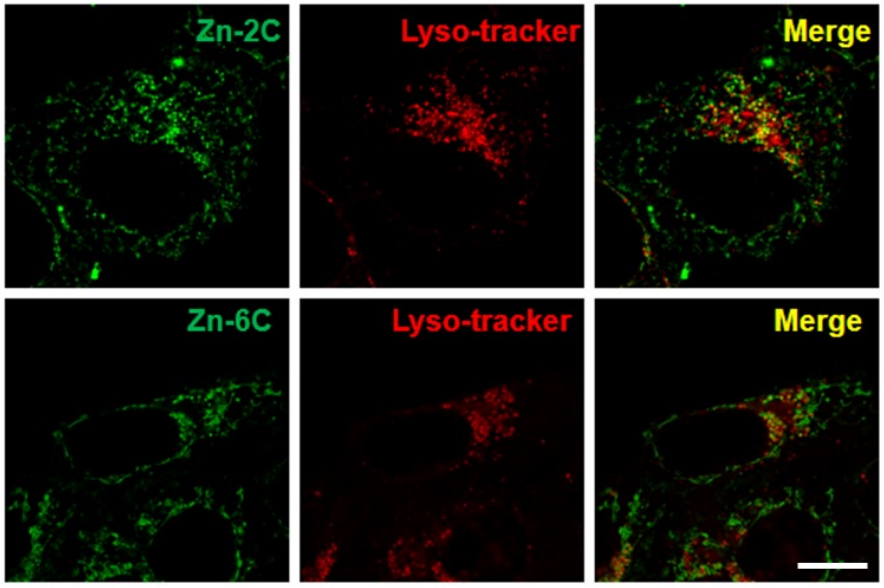


Figure S17 Co-localization of Zn-nC (1 μM, green channel) with Lyso-Tracker Red (0.5 μM, red channel) for 15 min. Scale bar = 20 μm. (Zn-nC: λ_{ex} = 405 nm, λ_{em} = 510 nm, Lyso-Tracker Red: λ_{ex} = 577 nm, λ_{em} = 590 nm).

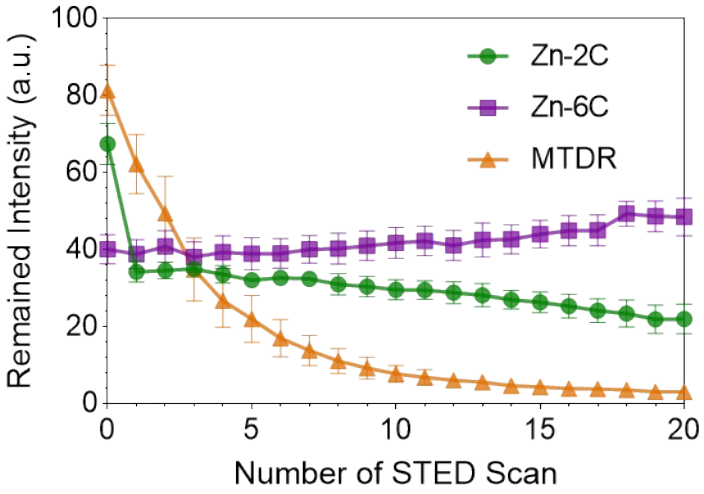


Figure S18 The STED photostability of Cell viability of Zn-nC (1 μM, 15 min) and commercial Mito-Tracker Deep Red (MTDR, 0.5 μM, 15 min) by monitoring their remained fluorescent intensity using a 595 nm (full power = 12 mW).

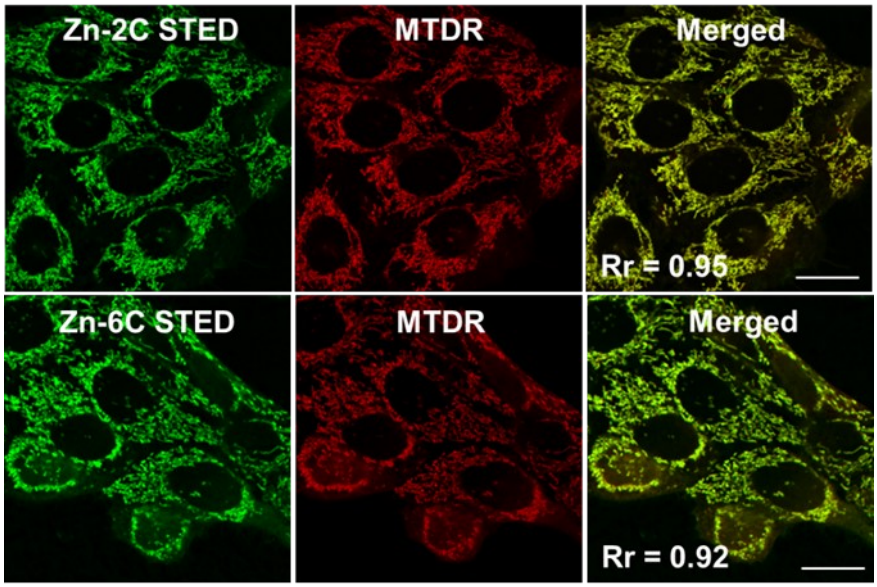


Figure S19 Co-localization of Zn-nC (1 μM, green channel) and commercial Mito-Tracker Deep Red (MTDR, red channel) (Zn-nC : Excitation = 405 nm, Emission = 480 - 550 nm, Depletion laser = 595 nm, full power = 12 mW; MTDR: Excitation = 633 nm, Emission = 640 - 650 nm. Donut laser = 660 nm, full power = 12 mW. Scale bar = 20 μm).

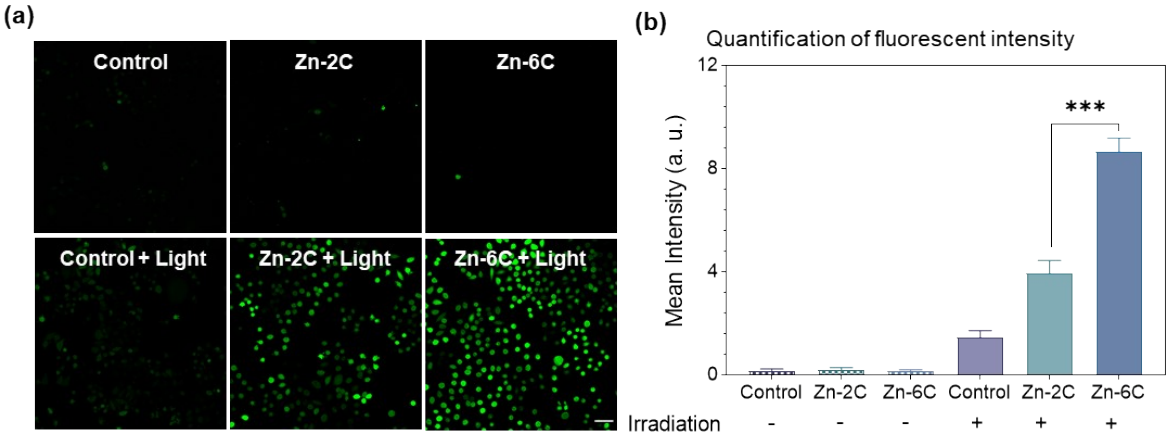


Figure S20 (a) Mitochondrial reactive oxygen species (ROS) were measured using Mitochondrial ROS assay kit under irradiation (405 nm lasers, 5 min, interval = 30 s). (b) The quantification fluorescent intensity analysis. Scale bar = 100 μm.

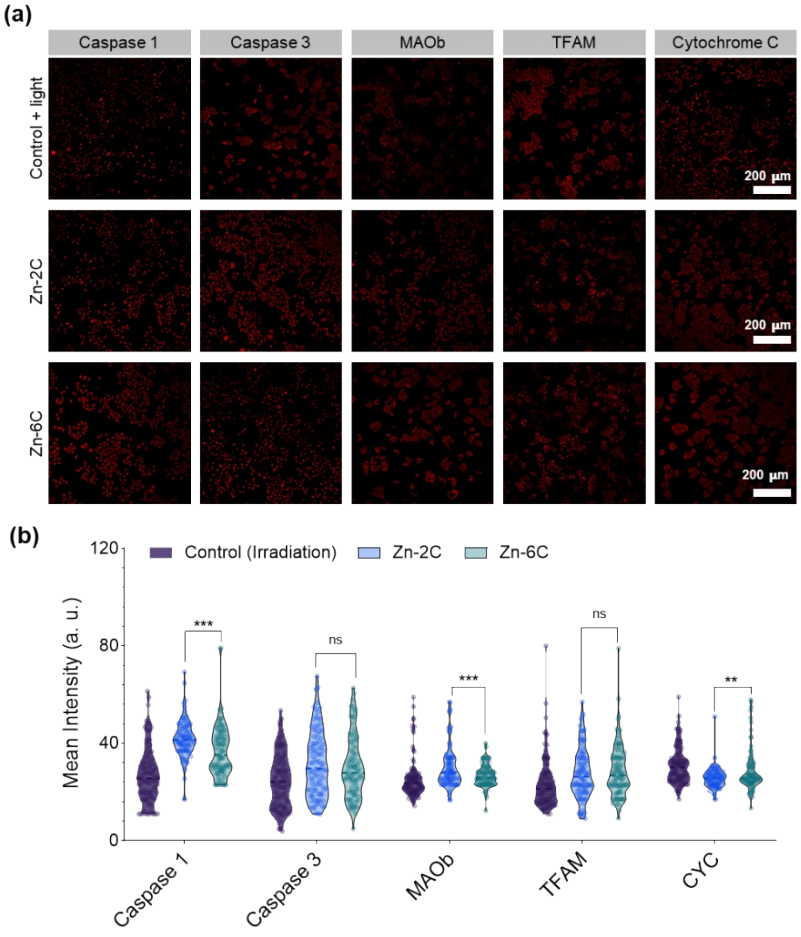


Figure S21 (a) Immunofluorescence analysis of Caspase 1, Caspase 3, MAOb, Cytochrome-C, and TFAM expression with Zn-nC (1 μM) treatment under ROS induced conditions (405 nm lasers, 5 min, interval = 30

s). (b) The average fluorescence intensity of 100 cells was selected from (a) for quantitative intensity analysis. (Secondary antibody: λex = 570 nm, λem = 590 nm). Error bar: SD, ***P < 0.001, **P < 0.01, *P < 0.05 and ns stands for no statistical significance were tested via t-test.

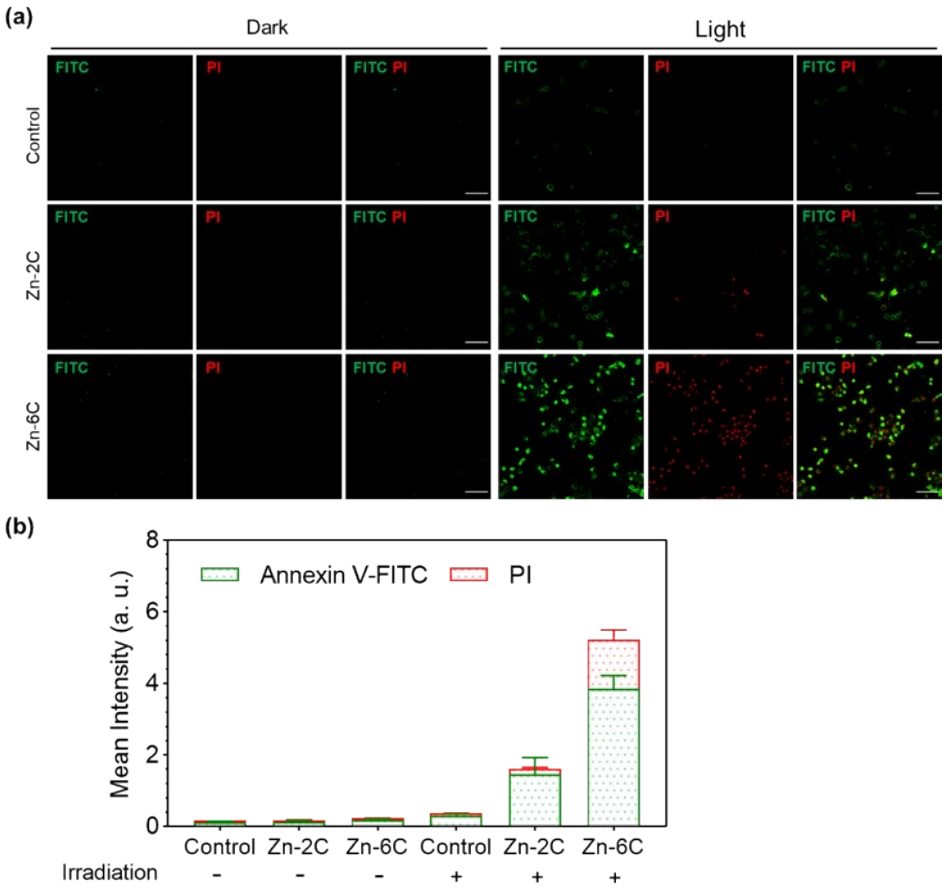


Figure S22 (a) Confocal fluorescence images of Annexin V–FITC/PI stained HeLa cells with Zn-nC (1 μM) treatment with or without ROS induced conditions (405 nm lasers, 5 min, interval = 30 s). (b) The quantification mean fluorescent intensity analysis of Figure a. Zn-nC : Excitation = 405 nm, Emission = 480 - 550 nm. Annexin V–FITC : Excitation = 488 nm, Emission = 500 - 550 nm. PI : Excitation = 561 nm, Emission = 570-700 nm. Scale bars: 100 μm.

1. B. Ni, H. Z. Cao, C. K. Zhang, S. L. Li, Q. Zhang, X. H. Tian, D. D. Li, J. Y. Wu and Y. P. Tian, *Inorg. Chem.*, 2020, **59**, 13671-13678.