Electronic Supplementary Information

Tuning the Organelle-Imaging Specificity of Aggregation-Induced Emission Luminogen with Reversible Mechanochromism by Ionization

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Content

I. Experimental Section

Chemical Reagents and Materials

4-(1,2,2-Triphenylvinyl)benzaldehyde[1] and 2-(4-bromo phenyl)-3-(4-(1,2,2-triphenylvinyl) phenyl) acrylonitrile[2] were prepared according to the literature methods. 2-(4-Bromophenyl) acetonitrile, quinoline-4-ylboronic acid, tetrakis(triphenylphosphine)palladium(0), Aliquat 336, anhydrous potassium carbonate, iodomethane, and potassium hexafluorophosphate were purchased from *Innochem*, *Energy Chemical* or *J&K* Scientific Ltd and used as received. All other analytical grade solvents and reagents were purchased from *Zeyuan* Company (Guangzhou, China) and used without further purification.

Measurements

^{1}H NMR and ^{13}C NMR were carried out on a Bruker AVANCE 400 MHz or 600 MHz spectrometer by using tetramethylsilane as internal standard and DMSO-d_6 as solvent. Mass spectra dates were gained by the instrument of LTQ Orbitrap LCMS (LTQ Orbitrap Elite). UV-vis absorption spectra were measured by using a Hitachi UV-3900H spectrophotometer. Absolute PL quantum yields and lifetimes of the samples were measured with an Edinburgh FLS980 spectrometer which is equipped with a calibrated integrating sphere. Photoluminescent spectra were achieved via an Ocean Optics spectrophotometer (QE65 Pro). Photoluminescent images what demonstrated the mechanochromic phenomena were taken by a Canon T5i digital camera with a Canon 50 mm f/1.8G STM camera lens. Powder XRD patterns were performed by an X-ray diffractometer (Rigaku, Ultima IV) at 293 K in a scanning rate of 5° (2θ)/min. Thermal behaviors for the samples were recorded by differential scanning calorimetry (DSC) at a heating rate of 10 ^{o}C/min under nitrogen atmosphere using a TA Q20 thermal analyzer.

Theoretical calculations

Density functional theory (DFT) calculation at the level of M06-2X/6-31G(d,p) was conducted by using Gaussian 16 software package.[3]

Cell Culture

A549 cells were purchased from Shanghai Cell Bank of the Chinese Academy of Sciences (China). A549 cell line was cultured in a medium (Dulbecco's Modified Eagle Medium (DMEM basix 1×) at 37 °C under a water-saturated atmosphere with 5% CO_2 (Series II water jacket CO_2 incubator, Thermo Fisher Scentific Gibco) , containing 10% fetal bovine serum (FBS，Gibco).

Biocompatibility

The biocompatibilities of PNOy and PNO were evaluated by Cell Counting Kit-8 (CCK8, DOJINDO). CCK8 medium mixture was prepared by mixing with 10 times of culture media in volume, firstly. Then, the nanosuspensions of PNOy and PNO were diluted by using DMEM complete medium. And the final mixtures of the AIEgens were added into A549 cell monolayers

(~5×10^4) in 96-well plates with different resulting concentrations and were incubated at 37 °C for 4 h, respectively. The CCK8 medium mixture (100 μL/well) was added to the plates and incubated at 37 °C for 1.5 h after washing with DPBS for three times. Finally, the absorbance values of the samples at 450 nm were measured with a microplate reader (BioTek EPOCH2 microplate reader, BioTek insruments Inc).

Bioimaging

A549 cells were seeded into a 35 mm glass bottom dish (Nunc) which bottom diameter is 27 mm. A549 cells were incubated with PNOy or PNO (10 μM) for 2 h, bodipy (Thermo Fisher, 200 nM) of PNO and MitoTracker Green (Thermo Fisher, 200 nM) of PNO for 45 min. After washing with DPBS for three times, they were imaged. Confocal imaging was performed by Zeiss 710 NLO with single-photon (λ_{ex} = 405 nm) imaging, and intense fluorescent signals were observed in the cytoplasm of the live A549 cells.

II. Synthesis and Structure Characterization

Synthetic procedures of the Compounds

Synthesis of PNOy 2-(4-bromophenyl)-3-(4-(1,2,2-triphenylvinyl)phenyl)acrylonitrile (1.00 g, 1.86 mmol), quinoline -4-ylboronic acid (0.40 g, 2.31 mmol), potassium carbonate (0.64 g, 4.64 mmol, 2 M aqueous solution), $Pd(PPh_3)_4$ (10.00 mg, 0.01 mmol) and THF (30 mL) were added into a three-necked flask. Then the mixture was degassed and stirred at 75 °C under argon for 36 h. The mixture was concentrated after cooling to room temperature. The resulting crude product was purified by silica gel column chromatography using DCM/ethanol (v/v = 55:1) as eluent. The solid was further recrystallized from DCM/ethanol to get a green powder in 63% yield. (0.69 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99-8.92 (d, J = 4.4 Hz, 1H), 8.15-8.07 (d, J = 8.4 Hz, 1H), 8.07-8.01 (s, 1H), 7.95-7.89 (d, J = 8.1 Hz, 2H), 7.89-7.84 (d, J = 8.4 Hz, 1H), 7.83-7.77 (m, 1H), 7.77-7.72 (d, J = 8.2 Hz, 1H), 7.71-7.64 (d, J = 8.1 Hz, 2H), 7.63-7.58 (t, J = 7.6 Hz, 1H), 7.52-7.46 (d, J = 4.5 Hz, 1H), 7.24-7.05 (m, 11H), 7.05-6.93 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 150.12, 148.10, 146.37, 145.94, 142.84, 142.64, 141.78, 139.69, 137.95, 134.14, 131.66, 131.25, 130.64, 130.20, 129.53, 128.81, 127.94, 127.80, 127.11, 126.89, 126.72, 126.11, 125.62, 125.22, 121.41, 117.85, 108.92.ESI-MS m/z: $[M+H]^+$ calcd. for, 587.2472; found, 586.2409.

Synthesis of PNO PNOy (0.20 g, 0.34 mmol), iodomethane (0.30 mL), and acetonitrile (20 mL) were added into a 250 mL three-necked flask. The mixture was heated to reflux and stirred for 18 h. After cooling to room temperature, the reaction mixture was poured into diethyl ether, and red precipitate was obtained. Subsequently, the red precipitate, acetone and saturated KPF_6 solution (10 mL) were added into a three-necked flask. After stirring at room temperature for 1 h, the

precipitate was then filtered and washed with water for several times to gain an orange solid powder. The powder was further recrystallized from acetone/water to get a yellow powder in 71% yield (0.18 g). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.57-9.51 (m, 1H), 8.62-8.58 (d, J = 8.8 Hz, 1H), 8.36-8.32 (m, 1H), 8.28-8.25 (d, J = 8.5 Hz, 1H), 8.22-8.19 (m, 1H), 8.15-8.12 (s, 1H), 8.09-8.03 (m, 3H), 7.86-7.83 (d, J = 8.5, 2H), 7.83-7.78 (d, J = 8.5, 2H), 7.23-7.13 (m, 11H), 7.08- 6.99 (m, 6H), 4.671-4.66 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 156.39, 148.99, 146.06, 143.63, 142.51, 141.69, 139.46, 138.58, 135.76, 135.12, 131.26, 130.62, 130.11, 128.77, 127.78, 126.71, 126.17, 121.99, 119.43, 117.50, 108.29, 45.07. ESI-MS m/z: [M-PF_6^-]$^+$ calcd. for, 601.2624; found, 601.2638.

^{1}H NMR, ^{13}C NMR, and MS of the Compounds

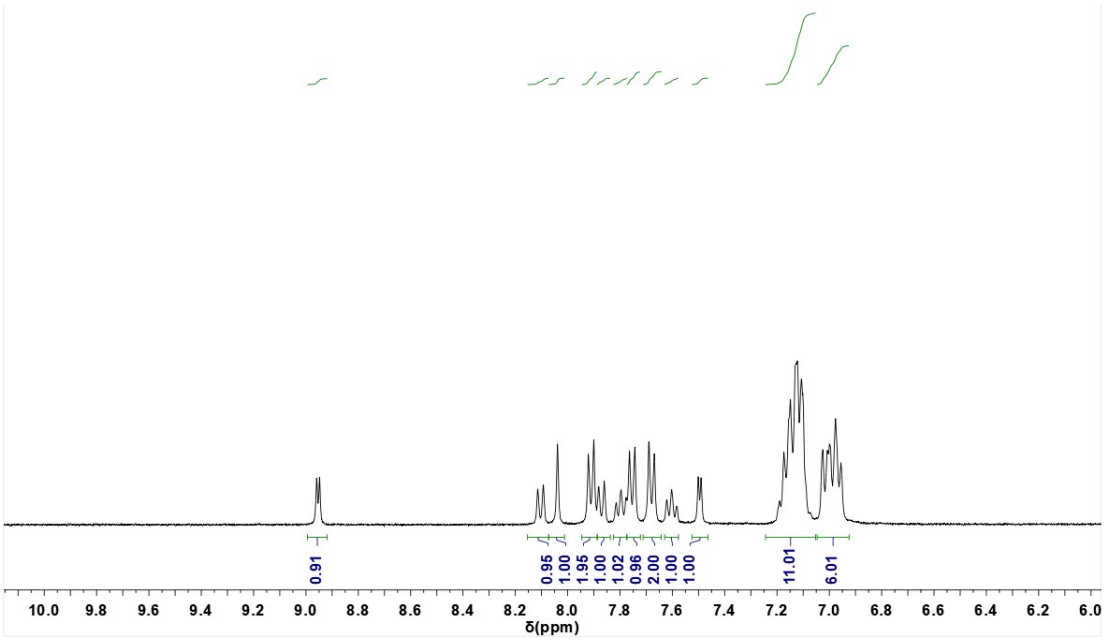

Figure S1. ^{1}H NMR spectrum of PNOy (in DMSO-d_6).

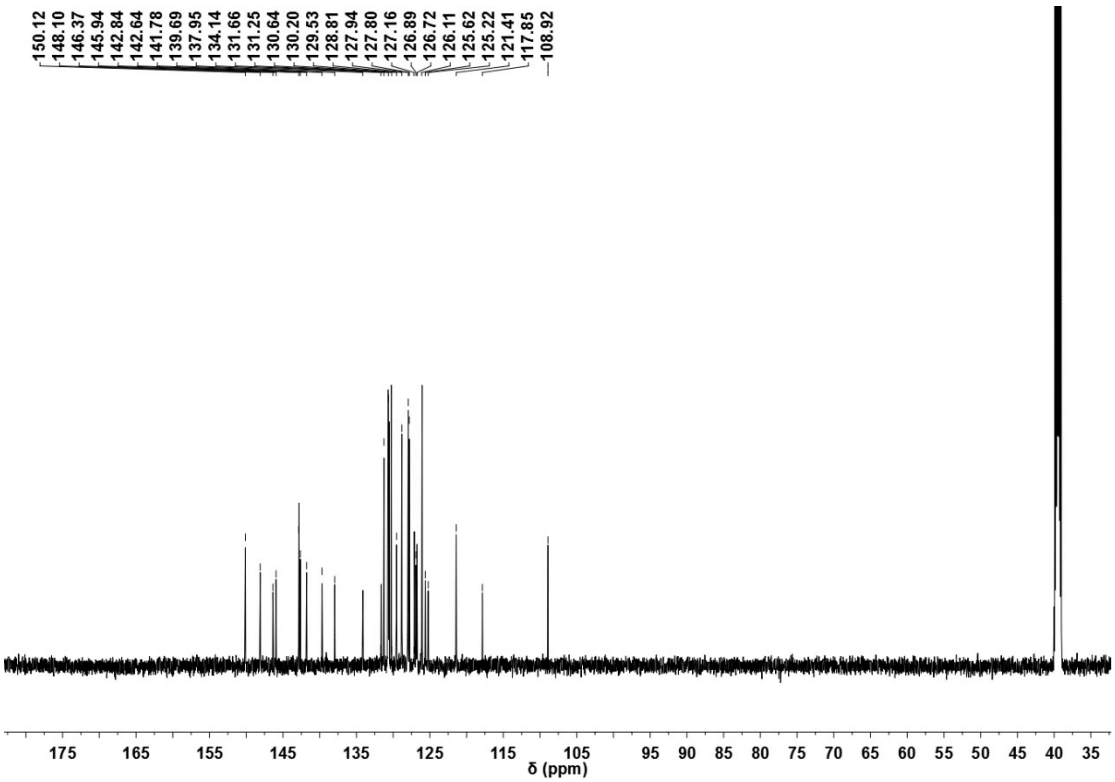

Figure S2. ^{13}C NMR spectrum of PNOy (in DMSO-d_6).

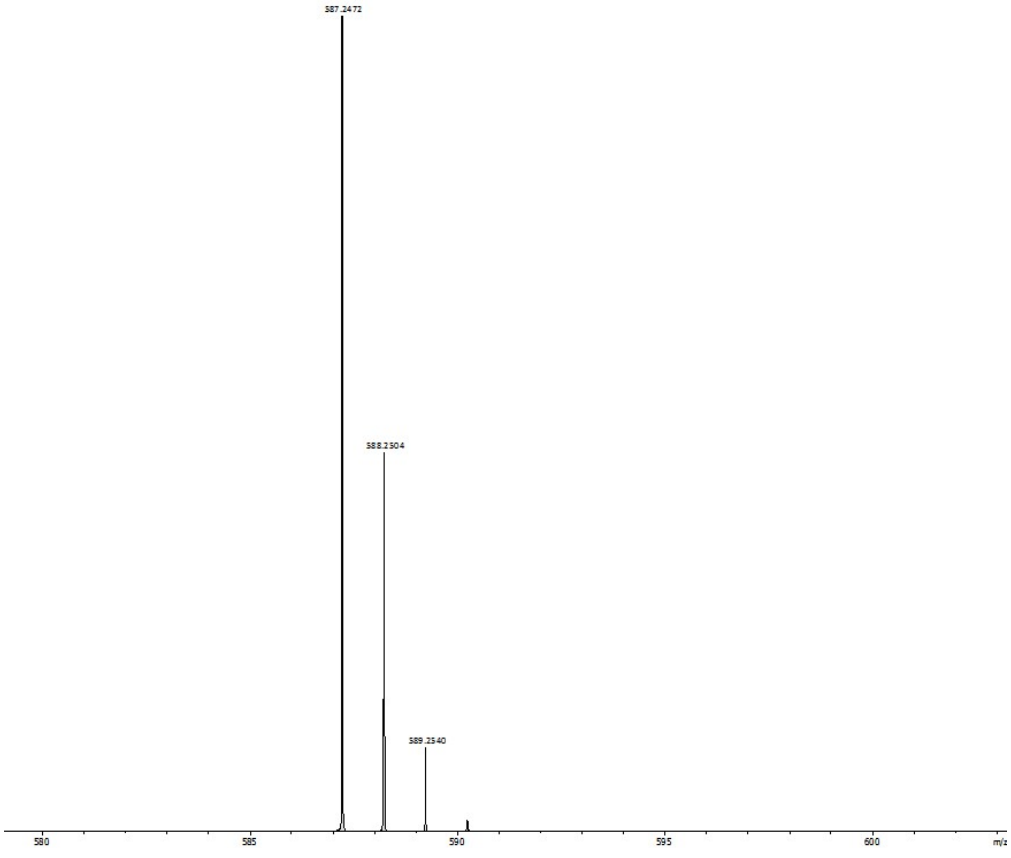

Figure S3. ESI-MS of PNOy.

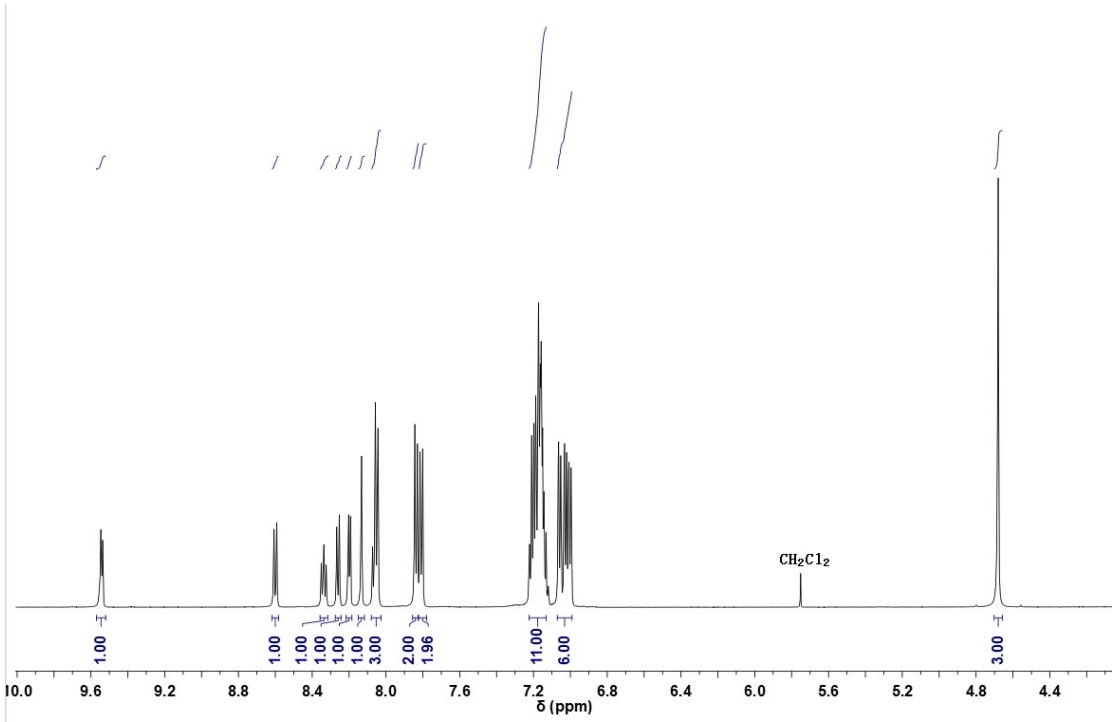

Figure S4. ^{1}H NMR spectrum of PNO **(**in DMSO-d_6**)**.

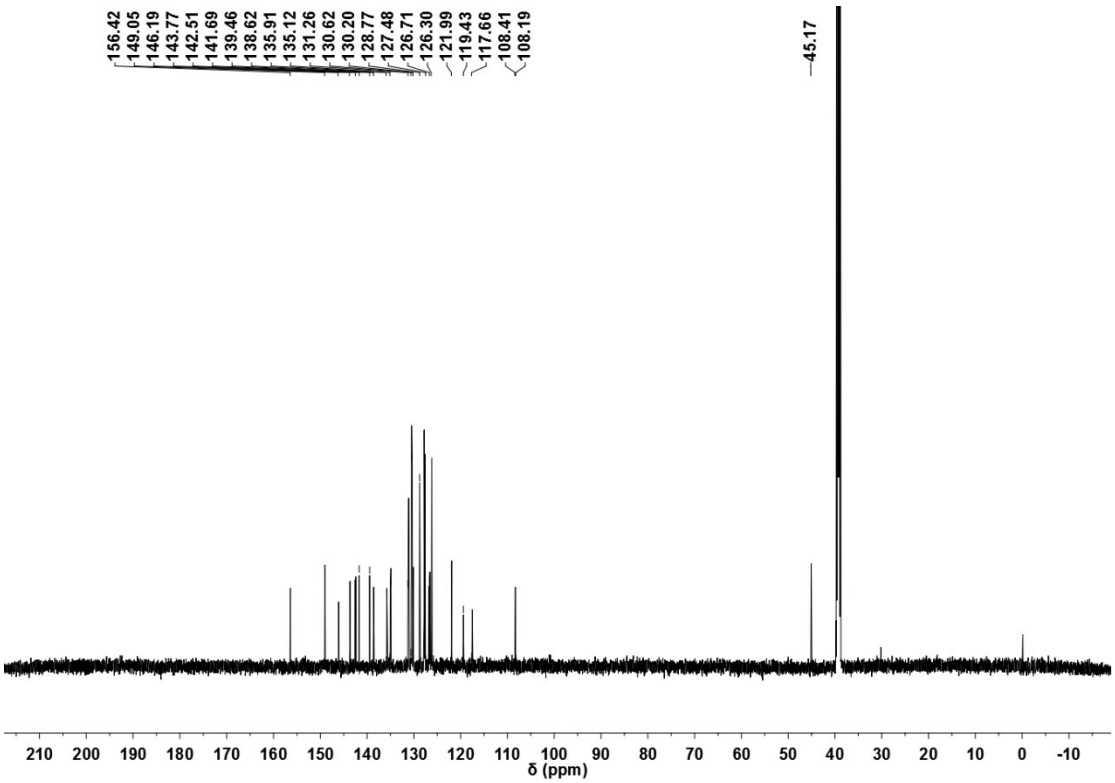

Figure S5. ^{13}C NMR spectrum of PNO **(**in DMSO-d_6**)**.

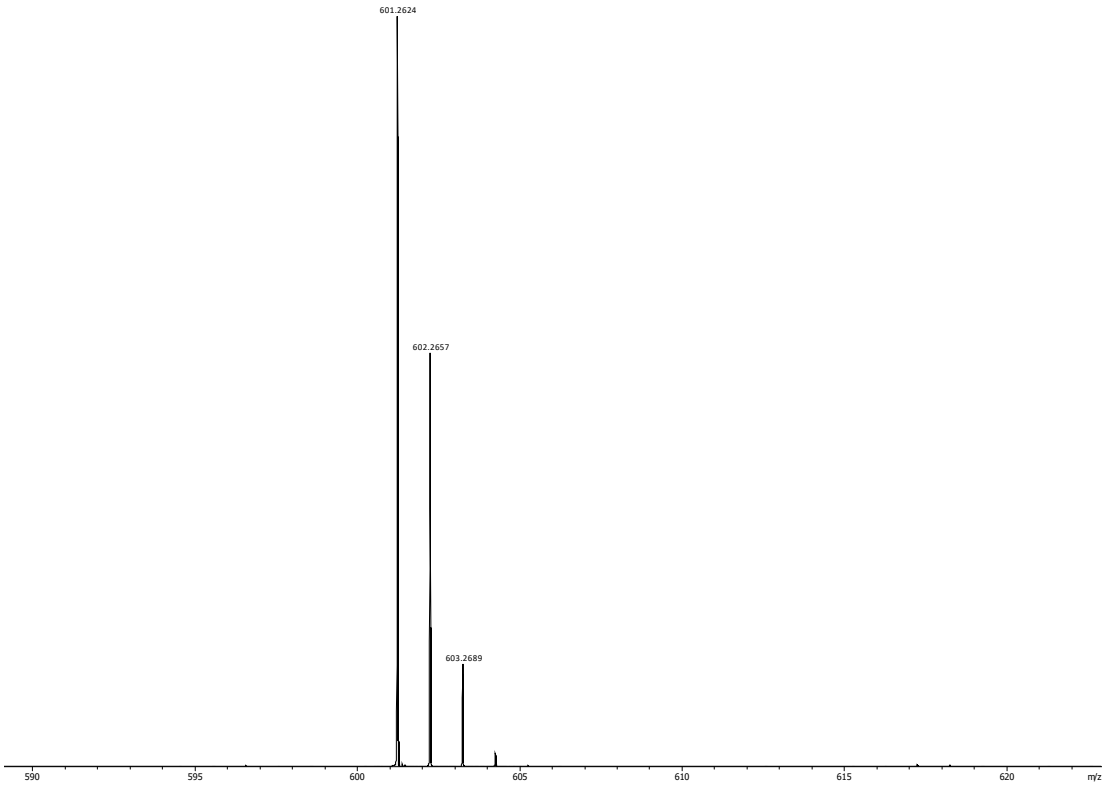

Figure S6. ESI-MS of PNO.

III. Photophysical and Morphological Properties of PNOy and PNO

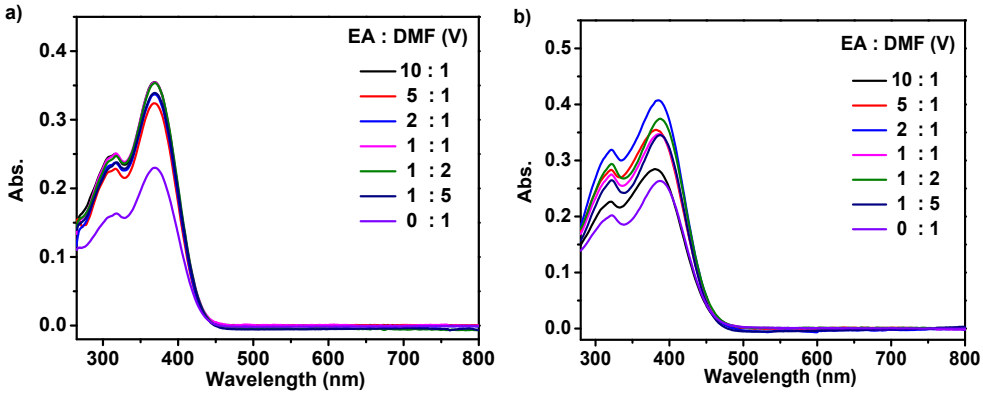

Figure S7. UV-visible absorption spectra of PNOy a) and PNO b) in the mixtures of ethyl acetate (EA) and *N*,*N*-dimethylformamide (DMF) with different volume ratios. (Concentration: 10 μM).

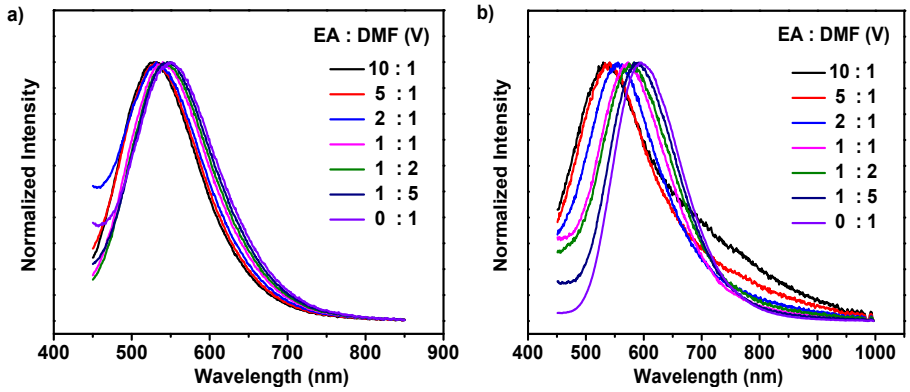

Figure S8. PL spectra of PNOy a) and PNO b) in the mixtures of EA and DMF with different volume ratios. (Concentration: 10 μM).

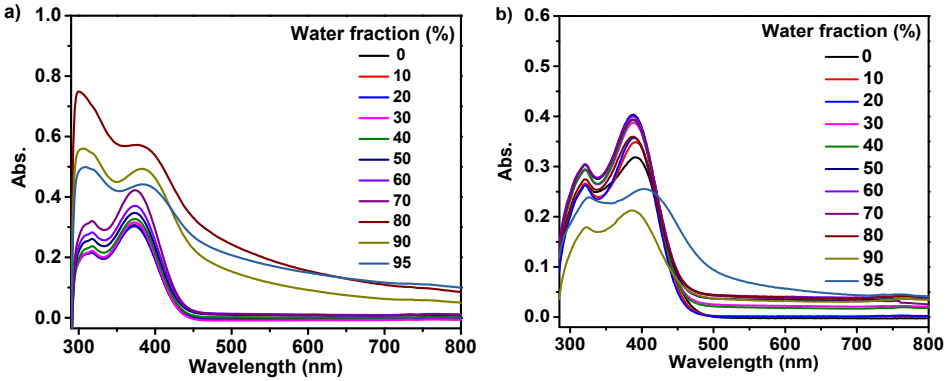

Figure S9. AIE properties of the compounds. UV-visible absorption spectra of PNOy a) and PNO b) in water/THF mixtures with different water fractions. (Concentration: 10 μM).

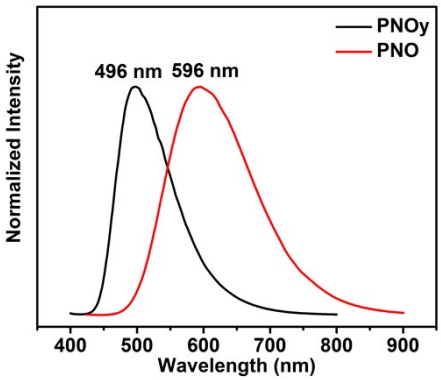

Figure S10. PL spectra of PNOy and PNO in solid states (excitation wavelength: 365 nm).

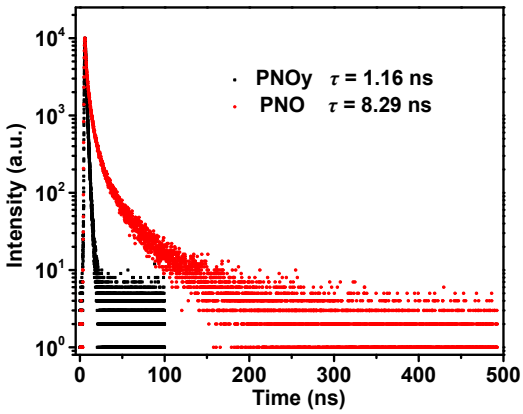

Figure S11. Emission decay curves of the pristine samples of PNOy and PNO.

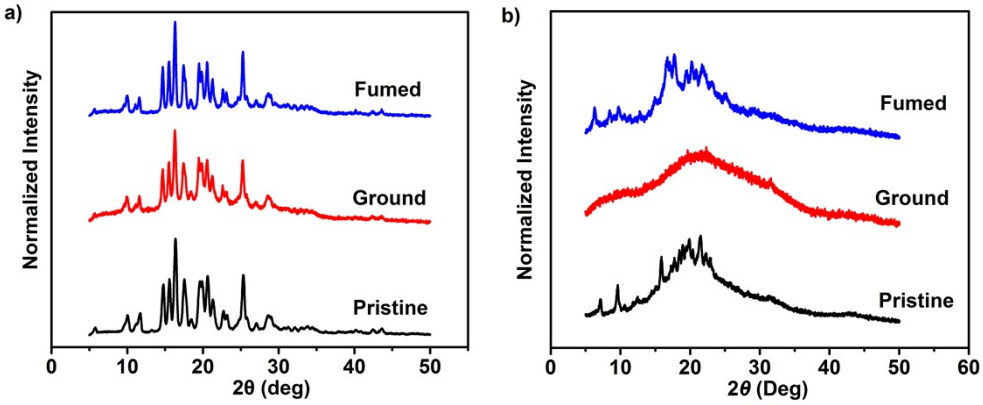

Figure S12. XRD patterns for the samples of PNOy a) and PNO b).

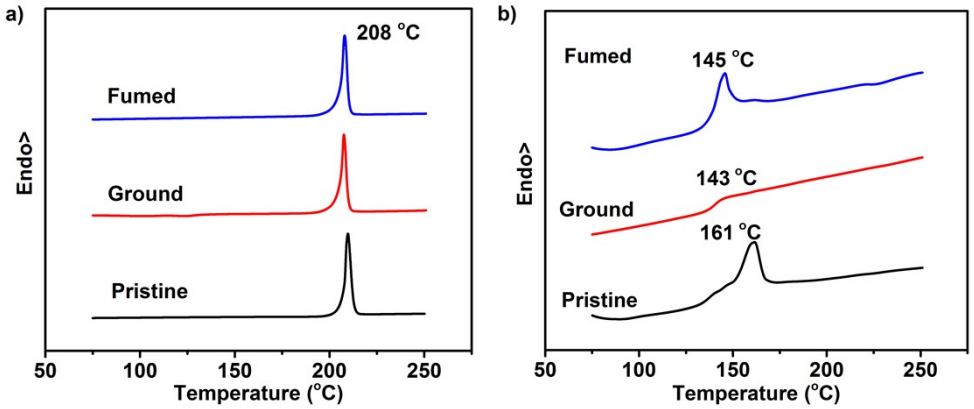

Figure S13. DSC curves for the samples of PNOy a) and PNO b).

Table S1. Mechanochromic properties of PNOy and PNO.

Compound	$\lambda_{em,p}$ (nm)[a]	$\Phi_{s,o}$ (%)[b]	$\lambda_{em,f}$ (nm)[c]	$\lambda_{em,g}$ (nm)[d]	$\Phi_{s,g}$ (%)[e]
PNOy	496	26.3	496	537	43.3
PNO	596	19.1	539	643	10.7

[a] Emission maximum of the original sample, [b] fluorescence quantum yield of the original sample, [c] emission maximum of the fumed sample, [d] emission maximum of the ground sample, [e] fluorescence quantum yield of the ground sample.

IV. In Vitro Bioimaging

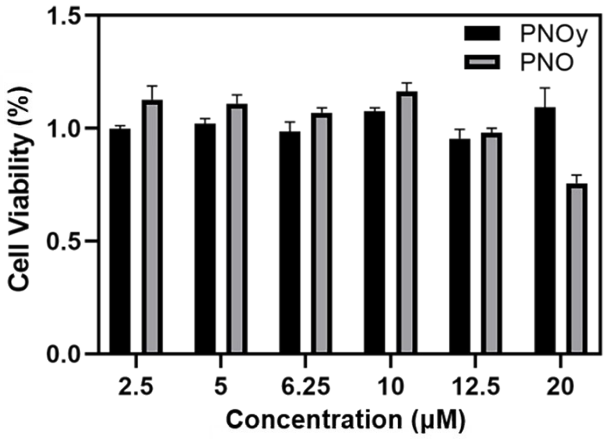

Figure S14. Cell viability with a series of doses of PNOy and PNO against A549 cancer cells for 4 hours by CCK8 assay.

References

1. B. Xu, M. Xie, J. He, B. Xu, Z. Chi, W. Tian, L. Jiang, F. Zhao, S. Liu, Y. Zhang, Z. Xu and J. Xu, *Chem. Commun.*, 2013, 49273-275.
2. X. Yang, Q. Wang, P. Hu, C. Xu, W. Guo, Z. Wang, Z. Mao, Z. Yang, C. Liu, G. Shi, L. Chen, B. Xu and Z. Chi, *Mater. Chem. Front.*, 2020, 4, 941-949.
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