Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supplementary Information for

Dihydropyridine-coumarin based fluorescent probe for imaging nitric oxide in living cells

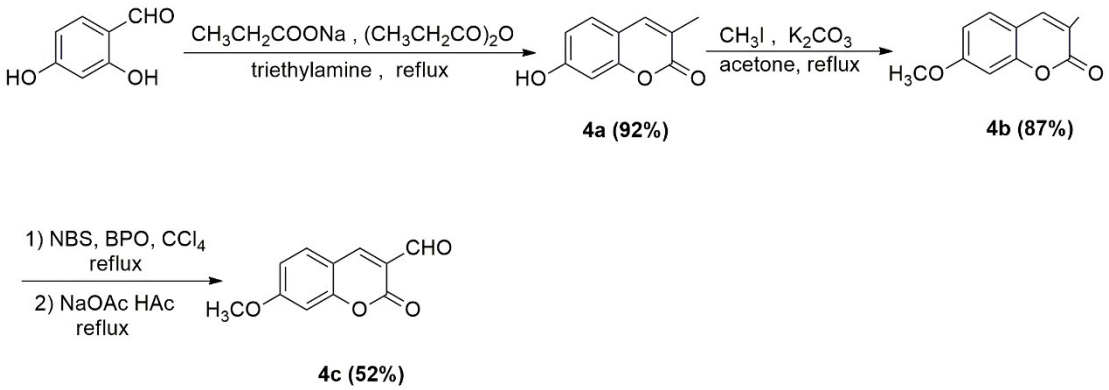
Sufang Ma[a,b*], Xueyi Sun[a], Qiang Yu[b], Rui Lui[a], Zhonglin Lu[a*], Lan He[a,c*]

[a] College of chemistry, Beijing Normal University, Beijing 100875, China. E-mail: masufang@sxmu.edu.cn

[b] Shanxi Medical University, Taiyuan 030000, China.

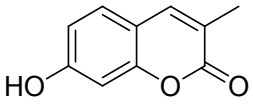
[c] National Institute for Food and Drug Control, Beijing 100050, China.

1. Synthesis of intermediate compounds



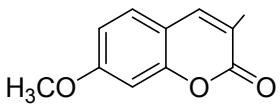
Scheme S1. Synthetic scheme

synthesis of 4a



2,4-Dihydroxybenzaldehyde (3.02 g, 26.6 mmol), sodium propionate (4.51 g, 46.8 mmol) and propionic anhydride (7.5 mL, 58.5 mmol) were added to the flask, and triethylamine (3.0 mL, 26.6 mmol) was added dropwise slowly with stirring. Then the mixture was refluxed for 12 h. The mixture was poured into water (100.0 mL), the crude product was precipitated, filtered, dried, and purified by chromatography (ethyl acetate/petroleum ether = 1:2). white solid was obtained (7.74 g, yield 92%). Mp. 210～212℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.58 (s, 1H)，7.57 (d, 1H, J = 8.0 Hz)，6.76 (d, 1H, J = 2.1 Hz)，6.62 (s, 1H)，2.43 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.5, 160.0, 154.4, 139.9, 128.5, 119.8, 112.9, 111.7, 101.8, 16.3; ESI-MS calculated 176.1, found 177.1 $[M+H]^+$. IR (KBr) υ 3416, 3092, 2980, 2925, 1763, 1717, 1612, 1417, 1354, 1134, 908, 759.

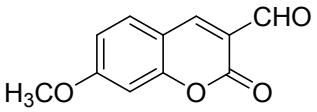
The synthesis of 4b



Potassium carbonate (4.03 g, 29.2 mmol) was added to the solution of **4a** (3.03 g, 14.6 mmol) in 50 mL of acetone, the mixture was stirred for 10 min. Then iodomethane

(3.12 g, 22.0 mmol) was added and refluxed for 6 h. The mixture was filtered at high temprature quklily, and the filtrate was concentrated. The crude product was recrystallized by ethanol to get white solid (3.03 g, 87%). Mp. 142～143℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H)，7.30 (d, 1H, *J* = 8.2 Hz)，6.83 (d, 1H, *J* = 2.4 Hz)，6.81 (s, 1H)，3.86 (s, 3H)，2.17 (d, 3H, *J* = 0.8 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.9, 178.0, 165.8, 160.6, 158.1, 145.9, 132.1, 118.4, 114.2, 112.0, 100.9, 56.1, 29.6. ESI-MS calculated 189.1, found 213.1 $[M+Na]^+$. IR (KBr) υ 2964, 2925, 2840, 1704, 1616, 1504, 1368, 1249, 1156, 1028, 833, 719.

The synthesis of 4c[1]



Under N_2 protection, N-bromosuccinimide (1.30 g, 7.3 mmol) and traces of benzoyl peroxide were added to the solution of **4b** (0.66 g, 3.5 mmol) in 20 mL of anhydrous carbon tetrachloride. After refluxing for 8 h, the solvent was removed, anhydrous sodium acetate (1.67 g, 20.8 mmol) and acetic acid (20 mL) were added to the mixture, and the mixture was refluxed for another 12 h. Hydrochloric acid (1.0 M, 10 mL) was added to the mixture and heated for 30 min continuously. After returning to room temperature, the mixture was stirred for 12 h. The solvent was evaporated, and the crude product was purified by chromatography (dichloromethane/petroleum ether = 1 : 1) to obtain yellow solid (0.32 g, yield 52%). Mp. 236～237℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.23 (s, 1H), 8.41 (s, 1H), 7.60 (s, 1H), 6.94 (d, 2H, *J* = 2.8 Hz), 3.59 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.7, 165.3, 159.4, 157.4, 147.0, 132.7, 118.1, 113.6, 111.8, 100.7, 56.3. ESI-MS calculated 204.2, found 204.1 $[M]^+$. IR (KBr) υ 3047, 2850, 1725, 1688, 1615, 1584, 1369, 1248, 1153, 1013, 856, 760.

2. recognition mechanism of DHP-4

A computational study was carried out using the Gaussian 09 program to understand the theoretical aspects of the change in fluorescence intensity. Density functional theory (DFT) calculations at the B3LYP/6-31+G** level were applied to obtain the optimized geometries and energy levels of frontier molecular orbitals of the components forming **DHP-4** and **PY-4** (7-methoxy-3-methyl-coumarin unit, dihydropyridine unit, and pyridine unit). As shown in Figure, the highest occupied molecular orbital (HOMO) of the dihydropyridine unit (electron donor) matches that of the 7-methoxy-3-methyl-coumarin unit (electron acceptor); therefore, when the latter is photoexcited, the intramolecular electron transfer from the dihydropyridine unit to the 7-methoxy-3-methyl-coumarin unit is energetically favorable. In contrast, the HOMO of the pyridine unit is significantly lower than that of the 7-methoxy-3-methyl-coumarin unit; consequently, the intramolecular electron transfer from the former to the latter isenergetically unfavorable. The DFT calculations indicate that the behavior of the NO turn-on fluorescence of DHP-4 is associated with the PET phenomenon.

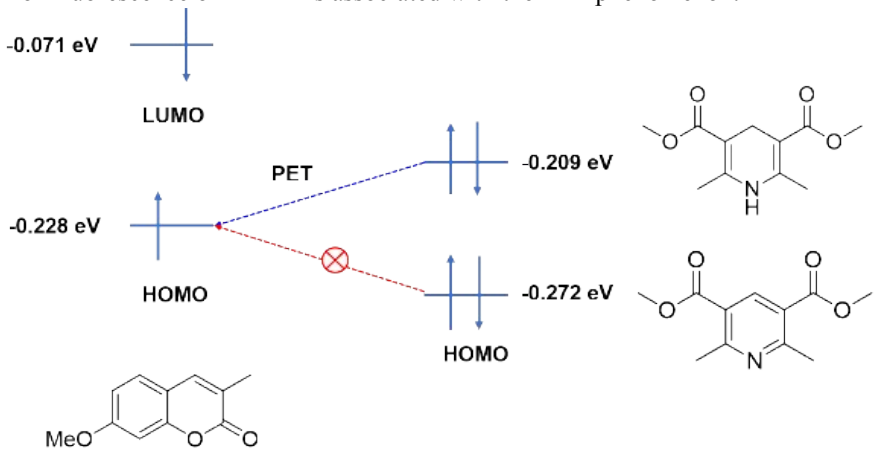


Fig.S1. The energy levels of frontier molecular orbitals of 7-methoxy-3-methyl-coumarin unit, dihydropyridine unit, and pyridine unit

3. **The characterization of the DHP-4 and PY-4**

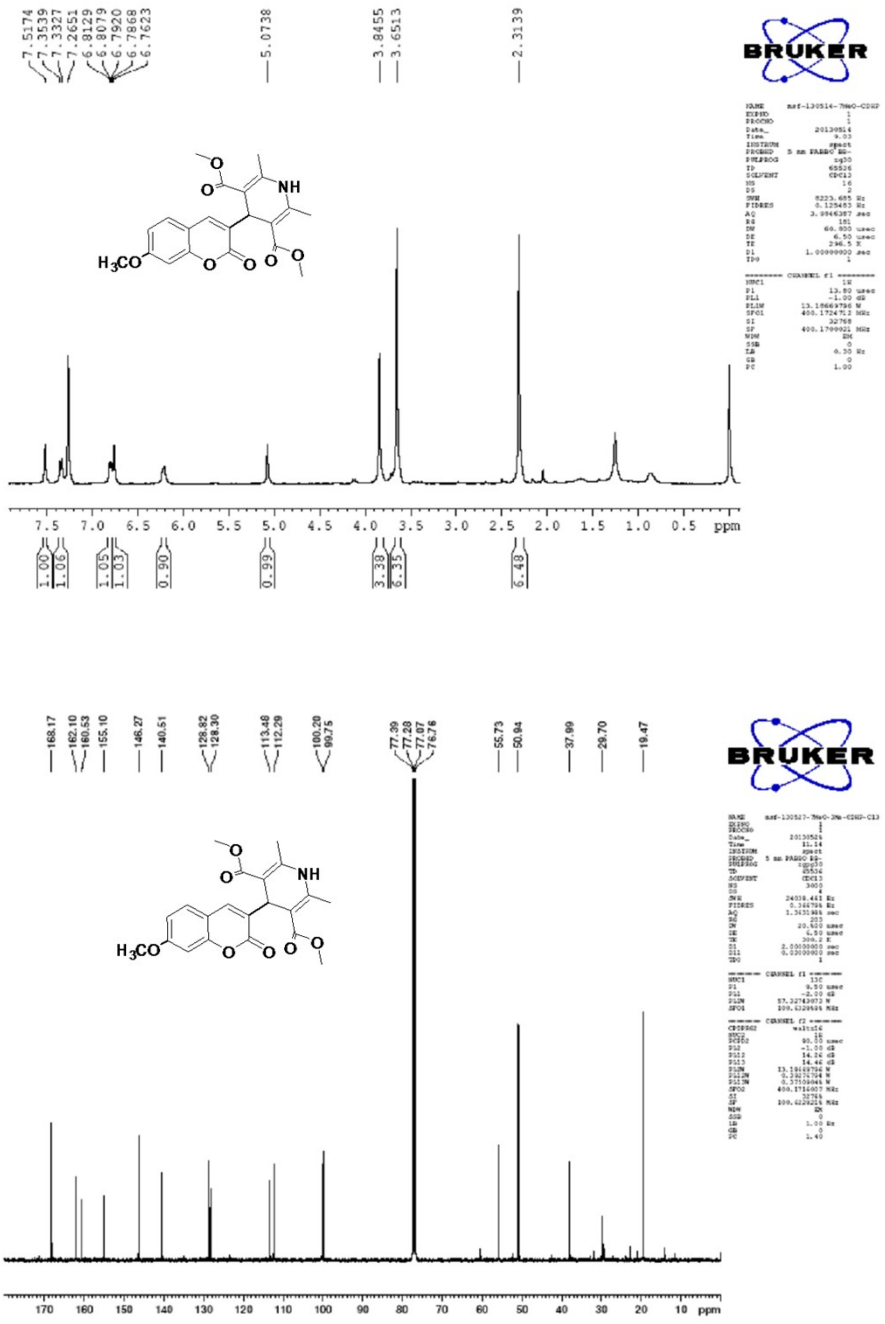


Fig.S2. ^{1}H NMR and ^{13}C NMR spectrum of **DHP-4** ($CDCl_3$, 400 MH_Z)

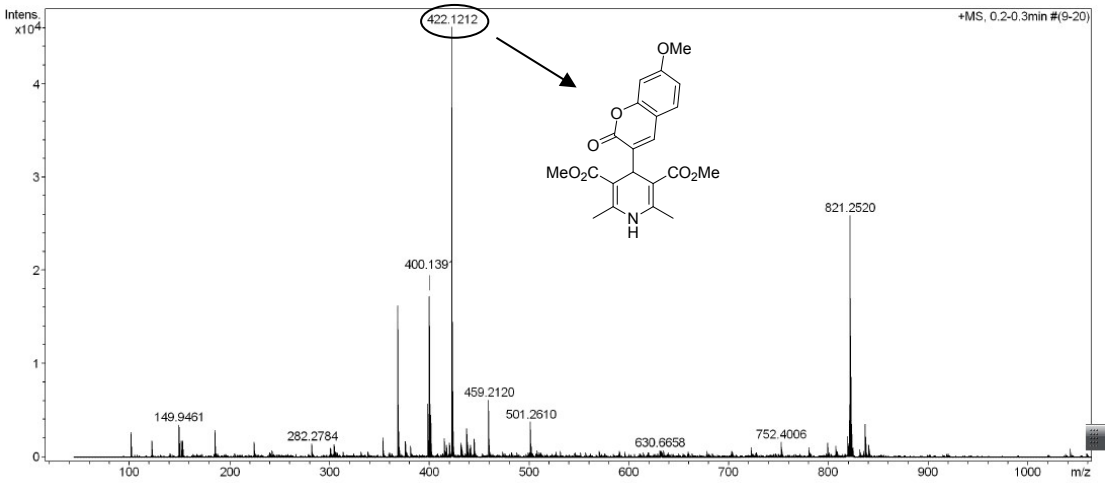


Fig.S3. HRMS of of **DHP-4**

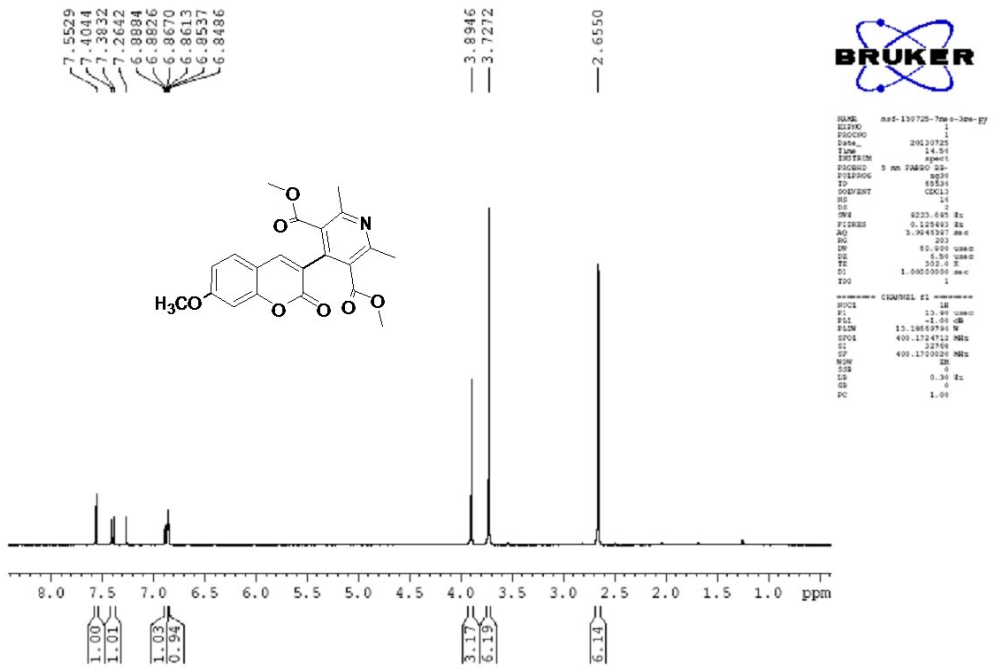


Fig.S4. ^{1}H NMR spectrum of **PY-4** ($CDCl_3$, 400 MH_Z)

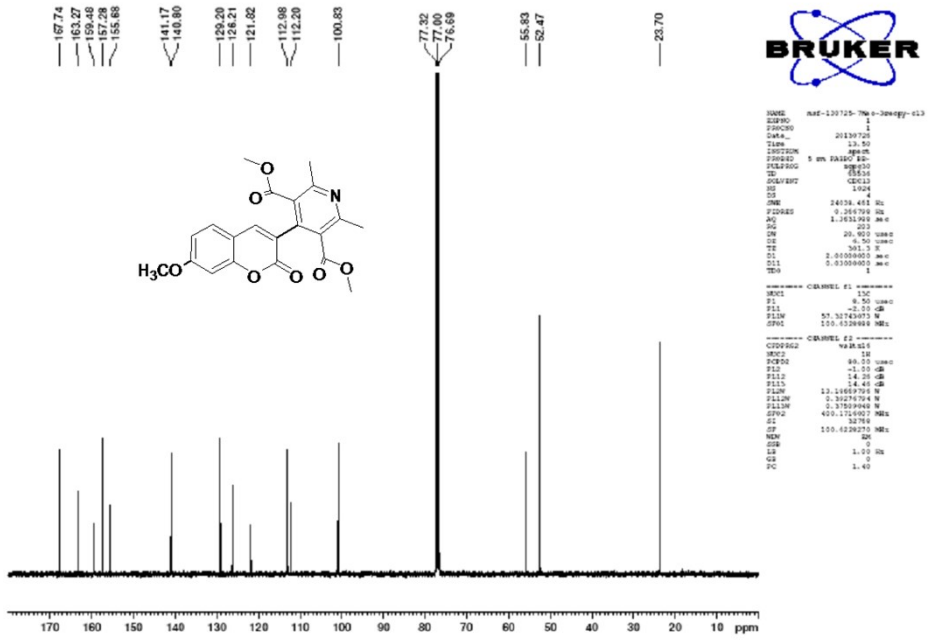


Fig.S5. ^{13}CNMR spectrum of **PY-4** ($CDCl_3$, 400 MH_Z)

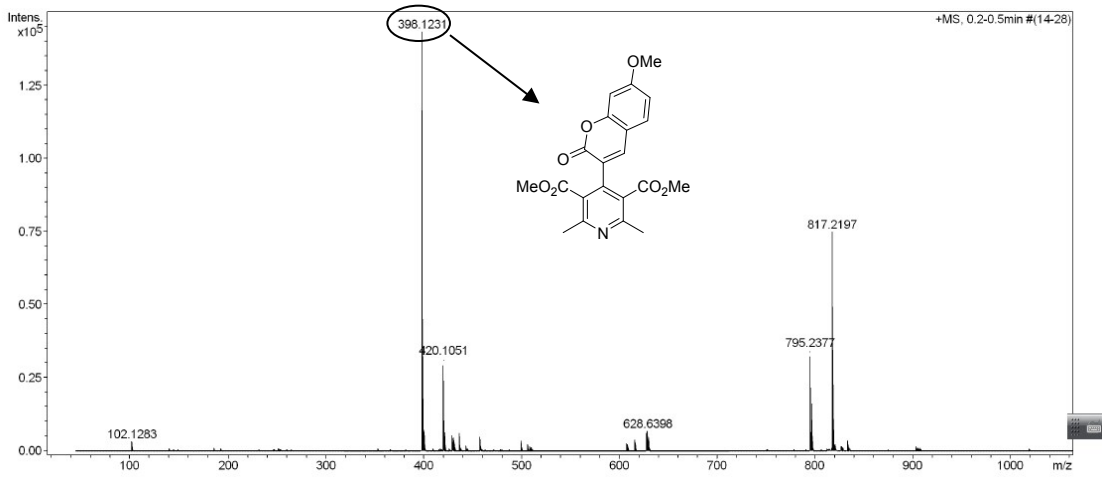


Fig.S6. HRMS of **PY-4**

References

[1] J. T. Hou, K. Li, B. Y. Liu, Y. X. Liao, X. Q. Yu, *Tetrahedron*, 2013, **69**, 2118-2123.