A Near-infrared Chemodosimeter with Pi-Selective Colorimetric and Fluorescent Sensing and Its Application in vivo Imaging

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General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel (230-400 mesh). ^{1}H NMR spectra were recorded using BRUKER DRX 500 spectrometer; ^{13}C NMR spectra were recorded using BRUKER DRX 500 spectrometer. The UV-Vis spectra were obtained using UV-240IPC spectrophoto meter. The fluorescence spectra were obtained with F-4500 FL spectrometer with a 1 cm standard quartz cell. Single-Crystal X-ray diffraction data was measured on Bruker-AXS SMART APEX II CCD The data collection was executed using the SMART program.

Confocal microscopy imaging

The images were observed with a fluorescence microscope (Olympus FV1000; Olymus, Tokyo, Japan) which was equipped with U-MWU2.

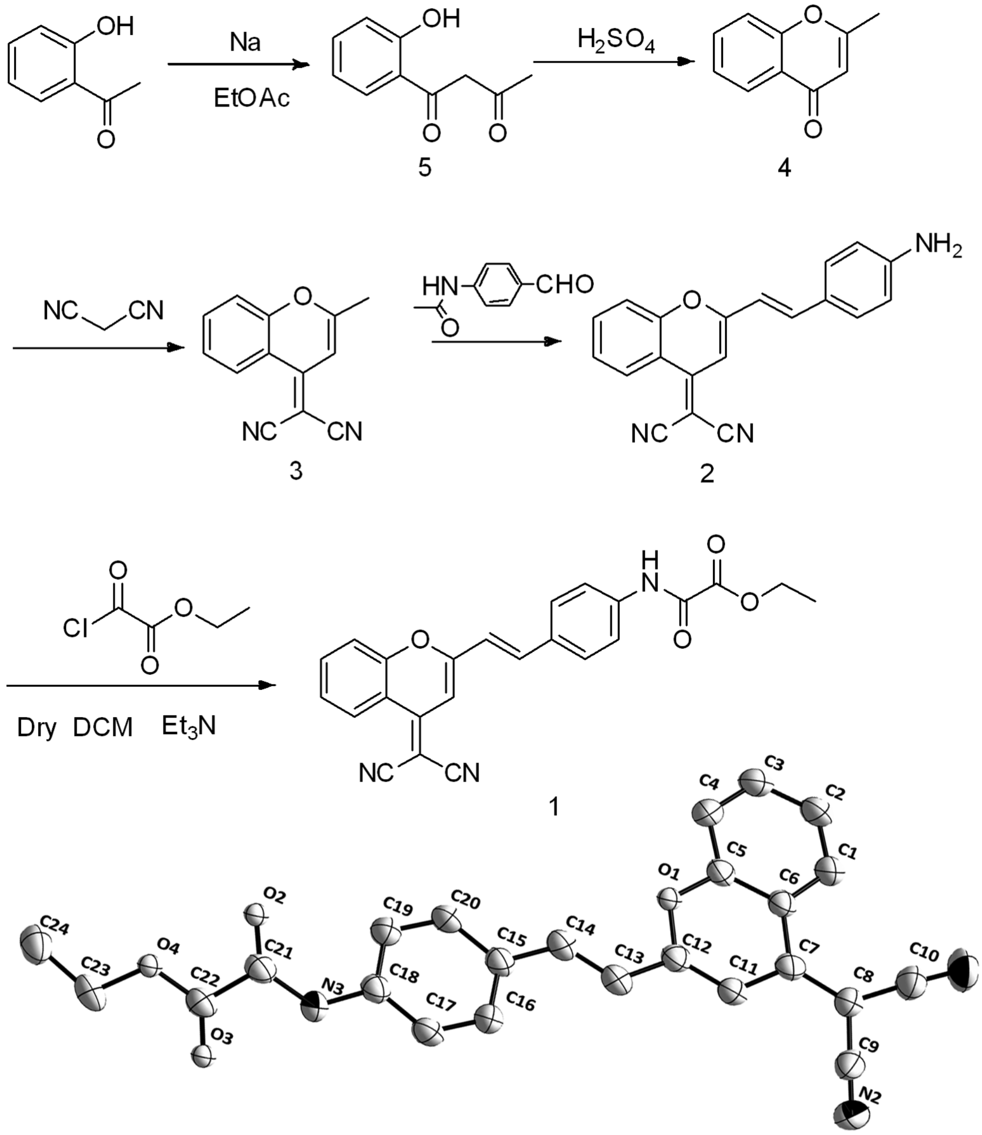
Culture of *C. elegans* and Fluorescent Imaging

The *C. elegans* wild type strain N2 was acquired from the Key Biological Laboratory Center (Yunnan University). The larval stage 4 (L4) *C. elegans* was used. The L4 stage nematodes were washed three times with deionized water by centrifugation at 2500 r/min for 2 minutes. For imaging of accumulations of PO_4^{3-} in the nematode, the previously exposed worms were incubated in centrifuge tube filled with 2 mL of deionized water, containing 20 μM of **1** at 20 °C for 1 h. The nematodes were washed three times again with deionized water by centrifuging at 2500 r/min for 2 minutes before being mounted onto a slide glass.

Culture of parameciums and Fluorescent Imaging

The parameciums was cultivated by ourselves. For imaging of accumulations of Pi in the parameciums, the previously exposed parameciums were incubated in small bottles filled with 20 mL of deionized water, containing 20 μM of **1** at 20 °C for 12 h. Then the parameciums were mounted onto a slide glass without further treatment. The images of the mounted nematodes were acquired by using Nikon ECLIPSE 90i fluorescence microscope using UV-2A Ex 330−380 (DM400, BA420) channel.

Synthesis and single-crystal data



1

Scheme S1. The synthesis route and single crystal of compound **1**.

Synthesis of 2-5

Compounds 2-5 were prepared according the later literature [1,2] with yields of 72.9 %, 81 %, 41.3 % and 24.7 %,, respectively.

Synthesis of 1

Under N_2 protection ,(E)-2-(2-(4-aminostyryl)-4H-chromen-4-ylidene) malononitrile (2) (153 mg, 0.5 mmol) and triethylamine (100 mg, 0.1 mmol) were dissolved in dry CH_2Cl_2, then ethyl 2-chloro-2-oxoacetate (250 mg , 2 mmol) was added to the sollution dropwised. The mixture was stirring at room temperature. After confirming the reaction was completed by TLC, the solvent was evaporated and the crude product was purified by silica column chromatography to give compound 5 (72 mg, 35.0 %), H^1 NMR: δ 10.990 (1 H, s), 8.76 (1 H, d), 7.91 (7 H, m), 7.71 (1 H, t), 7.60 (1 H, d), 7.03 (1 H, s), 4.34 (2 H, m), 1.33(3 H, t).

The CCDC number of probe 1 is 1032275.

The CIF file is put as a separated file in S. I.-2

Table S2. Bond lengths [A] and angles [deg] for 1.

N(1)-C(10)	1.143(4)
N(2)-C(9)	1.146(3)
N(3)-C(21)	1.360(3)
N(3)-C(18)	1.421(3)
O(1)-C(12)	1.351(3)
O(1)-C(5)	1.376(3)
O(2)-C(21)	1.212(3)
O(3)-C(22)	1.204(3)
O(4)-C(22)	1.316(3)
O(4)-C(23)	1.456(3)
C(1)-C(2)	1.372(4)
C(1)-C(6)	1.393(3)
C(1)-H(1)	0.9300
C(2)-C(3)	1.385(3)
C(2)-H(2)	0.9300
C(3)-C(4)	1.359(3)
C(3)-H(3)	0.9300
C(4)-C(5)	1.387(3)

C(4)-H(4)	0.9300
C(5)-C(6)	1.401(3)
C(6)-C(7)	1.459(3)
C(7)-C(8)	1.389(3)
C(7)-C(11)	1.429(3)
C(8)-C(10)	1.409(4)
C(8)-C(9)	1.426(4)
C(11)-C(12)	1.343(3)
C(11)-H(11)	0.9300
C(12)-C(13)	1.451(3)
C(13)-C(14)	1.334(3)
C(13)-H(13)	0.9300
C(14)-C(15)	1.447(3)
C(14)-H(14)	0.9300
C(15)-C(20)	1.392(3)
C(15)-C(16)	1.395(3)
C(16)-C(17)	1.375(3)
C(16)-H(16)	0.9300
C(17)-C(18)	1.388(3)
C(17)-H(17)	0.9300
C(18)-C(19)	1.381(3)
C(19)-C(20)	1.384(3)

C(19)-H(19)	0.9300
C(20)-H(20)	0.9300
C(21)-C(22)	1.534(3)
C(23)-C(24)	1.487(4)
C(23)-H(23A)	0.9700
C(23)-H(23B)	0.9700
C(24)-H(24A)	0.9600
C(24)-H(24B)	0.9600
C(24)-H(24C)	0.9600
C(21)-N(3)-C(18)	125.1(2)
C(12)-O(1)-C(5)	119.01(18)
C(22)-O(4)-C(23)	116.4(2)
C(2)-C(1)-C(6)	121.6(2)
C(2)-C(1)-H(1)	119.2
C(6)-C(1)-H(1)	119.2
C(1)-C(2)-C(3)	120.5(3)
C(1)-C(2)-H(2)	119.8
C(3)-C(2)-H(2)	119.8
C(4)-C(3)-C(2)	120.0(2)
C(4)-C(3)-H(3)	120.0
C(2)-C(3)-H(3)	120.0
C(3)-C(4)-C(5)	119.2(2)

C(3)-C(4)-H(4)	120.4
C(5)-C(4)-H(4)	120.4
O(1)-C(5)-C(4)	114.9(2)
O(1)-C(5)-C(6)	122.4(2)
C(4)-C(5)-C(6)	122.6(2)
C(1)-C(6)-C(5)	116.0(2)
C(1)-C(6)-C(7)	125.8(2)
C(5)-C(6)-C(7)	118.1(2)
C(8)-C(7)-C(11)	119.5(2)
C(8)-C(7)-C(6)	125.1(2)
C(11)-C(7)-C(6)	115.5(2)
C(7)-C(8)-C(10)	126.4(2)
C(7)-C(8)-C(9)	120.3(2)
C(10)-C(8)-C(9)	113.2(2)
N(2)-C(9)-C(8)	178.7(3)
N(1)-C(10)-C(8)	176.5(3)
C(12)-C(11)-C(7)	122.4(2)
C(12)-C(11)-H(11)	118.8
C(7)-C(11)-H(11)	118.8
C(11)-C(12)-O(1)	122.2(2)
C(11)-C(12)-C(13)	125.3(2)
O(1)-C(12)-C(13)	112.5(2)

C(14)-C(13)-C(12)	123.6(2)
C(14)-C(13)-H(13)	118.2
C(12)-C(13)-H(13)	118.2
C(13)-C(14)-C(15)	128.6(2)
C(13)-C(14)-H(14)	115.7
C(15)-C(14)-H(14)	115.7
C(20)-C(15)-C(16)	116.5(2)
C(20)-C(15)-C(14)	119.1(2)
C(16)-C(15)-C(14)	124.5(2)
C(17)-C(16)-C(15)	121.6(2)
C(17)-C(16)-H(16)	119.2
C(15)-C(16)-H(16)	119.2
C(16)-C(17)-C(18)	120.9(2)
C(16)-C(17)-H(17)	119.6
C(18)-C(17)-H(17)	119.6
C(19)-C(18)-C(17)	118.8(2)
C(19)-C(18)-N(3)	123.9(2)
C(17)-C(18)-N(3)	117.3(2)
C(18)-C(19)-C(20)	119.7(2)
C(18)-C(19)-H(19)	120.1
C(20)-C(19)-H(19)	120.1
C(19)-C(20)-C(15)	122.6(2)

C(19)-C(20)-H(20)	118.7
C(15)-C(20)-H(20)	118.7
O(2)-C(21)-N(3)	127.2(2)
O(2)-C(21)-C(22)	121.2(2)
N(3)-C(21)-C(22)	111.6(2)
O(3)-C(22)-O(4)	125.2(2)
O(3)-C(22)-C(21)	123.5(2)
O(4)-C(22)-C(21)	111.3(2)
O(4)-C(23)-C(24)	107.9(2)
O(4)-C(23)-H(23A)	110.1
C(24)-C(23)-H(23A)	110.1
O(4)-C(23)-H(23B)	110.1
C(24)-C(23)-H(23B)	110.1
H(23A)-C(23)-H(23B)	108.4
C(23)-C(24)-H(24A)	109.5
C(23)-C(24)-H(24B)	109.5
H(24A)-C(24)-H(24B)	109.5
C(23)-C(24)-H(24C)	109.5
H(24A)-C(24)-H(24C)	109.5
H(24B)-C(24)-H(24C)	109.5

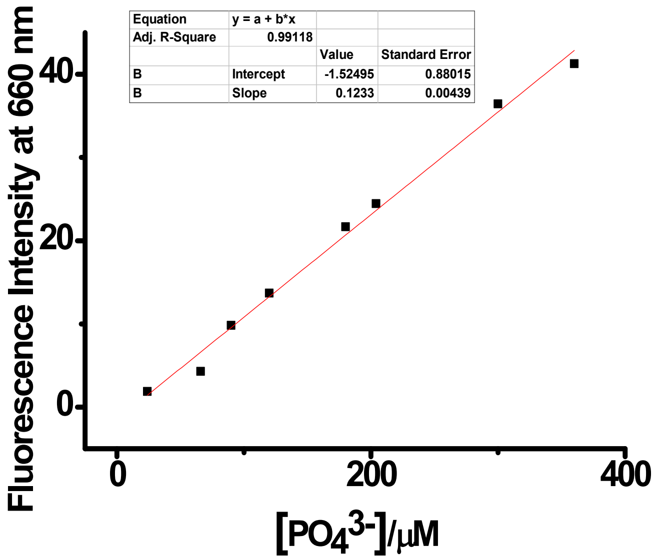


Figure S3. The changes in the fluorescence intensity of **1** (3.0 × 10^{-6} M) at 570 nm against varied concentrations of Pi from 0 to 400 μM in DMSO–HEPES buffer (0.02 M, pH = 7.0) (V/V = 9:1).

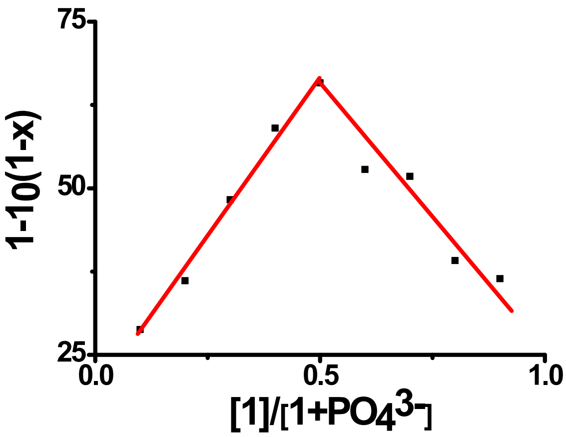


Figure S4. The job plot using fluorescent intensity at 580 nm of **1** (3 × 10^{-5} M) and Pi in DMSO–HEPES buffer (0.02 M, pH = 7.0) (V/V = 9:1). The total concentration of **1** and Pi was 100 μM.

H[1]NMR titration

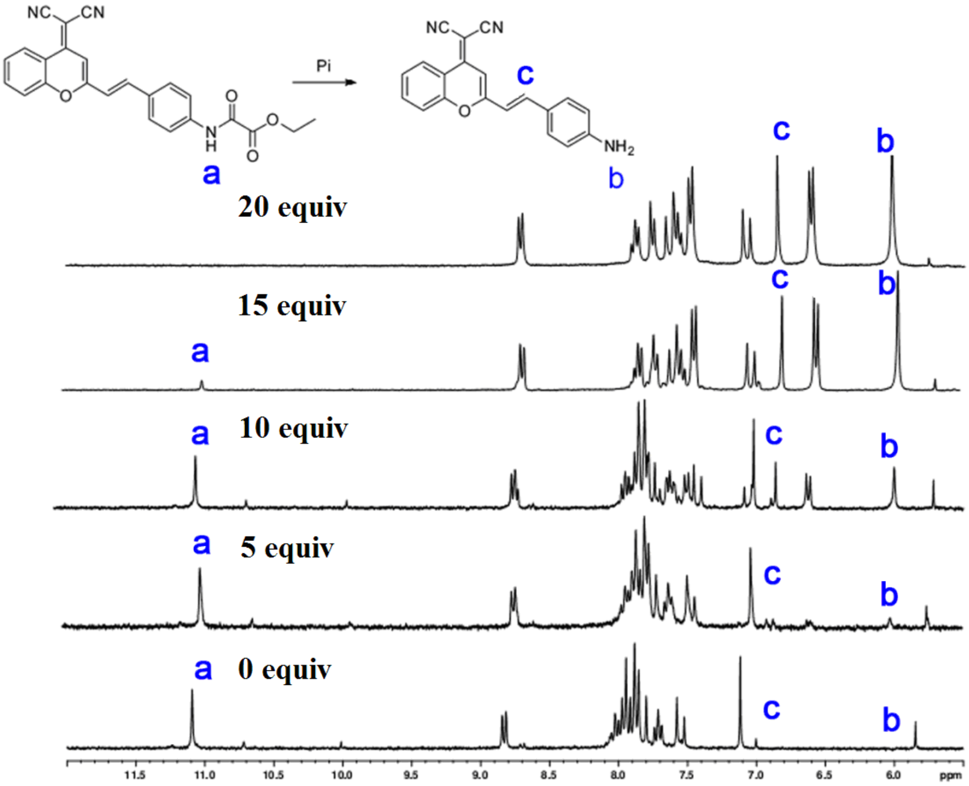


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Figure S5. [1]H NMR spectra of compound **1** (0.5 mM) with Pi in DMSO-*d6*; equivalents of Pi are related to the concentration of compound **1**.

HRMS

	Formula	Calculated m/z (amu)	mDa Error	PPM Error	DBE
1	C20 H12 N3 O	310.0985	-0.0857	-0.2764	16.5

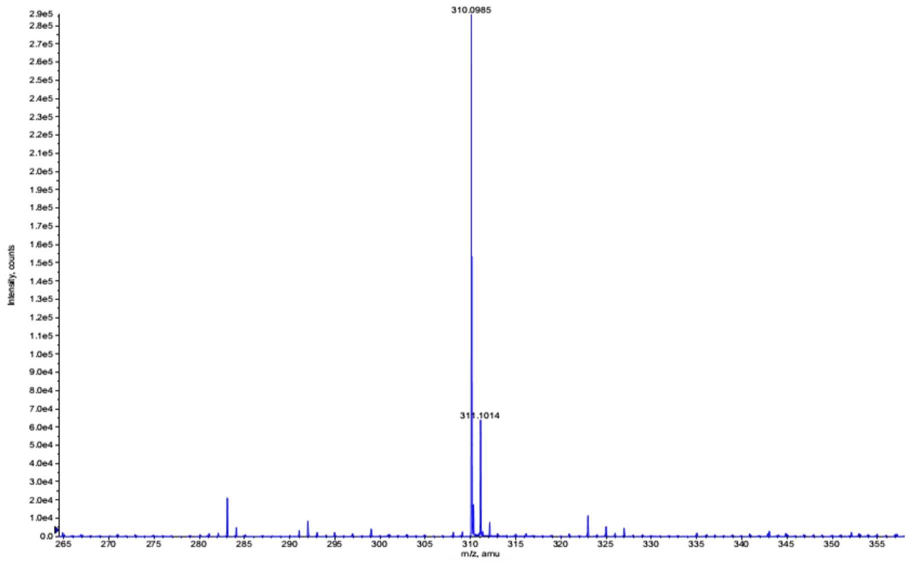

Figure S6. The HRMS spectra was recorded after 5 mg of probe **1** and 120 equiv of Pi (in 5ml DMSO-HEPES buffer (V/V=9:1)) stirred over night. HRMS (ESI): calcd for $C_{20}H_{12}NO_3$ $[M\ -H]^-$ =310.0985, found *m/z* 310.0985.

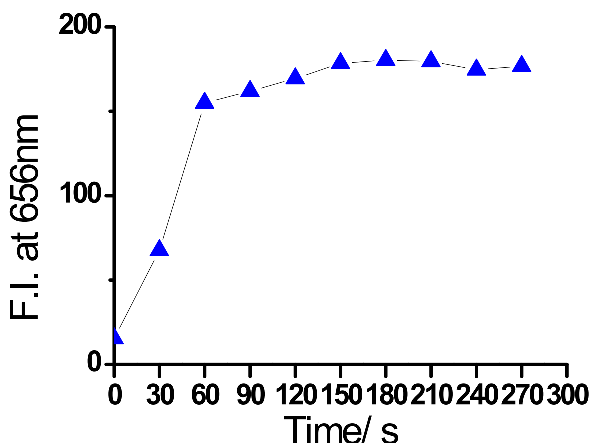

Figure S7. Time-trace plots of **1** (3×10^{-5} M) and Pi (6×10^{-3} M, monitored by the emission at 656 nm. (DMSO–HEPES buffer (20 mM, pH = 7.0)).

Reference:

(1) Sun, W.; Fan, J.; Hu, C.; Cao, J.; Zhang, H.; Xiong, X.; Wang, J.; Cui, S.; Sun, S.; Peng, X. *Chem. Commun.,* **2013**, *49*, 3890.

(2) Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun.*, **2011**, *47*, 8656.