Electronic Supplementary Information

for

Turn-On Two-Photon Fluorescent Probe for ATP and ADP

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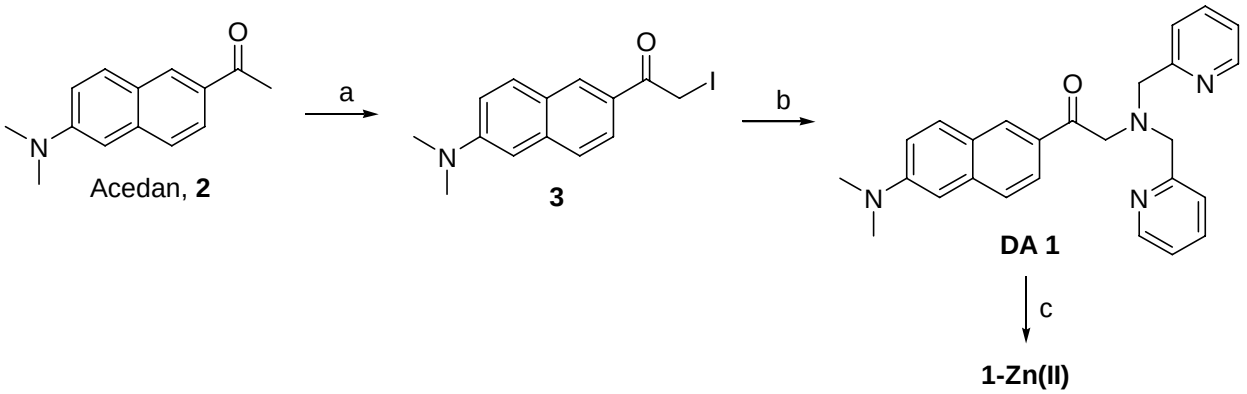
Materials and Methods

All chemicals were of reagent grade, purchased from Sigma-Aldrich and used without further purification. All the reactions were performed under argon atmosphere unless otherwise stated. Compounds purified by column chromatography were carried out on silicagel 60 (230-400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 spectrometer. NMR chemical shifts are reported in units of δ (ppm). UV/Vis spectra were recorded on an HP 8453 spectrophotometer. Fluorescence spectra were recorded on a Photon Technology International fluorometer with a 10 mm cuvette. Melting point was measured on Electrochemical MEL-TEMP $^{@}$3.0.

EXPERIMENTAL SECTION

Acedan was iodinated using iodine and LiHMDS in THF to afford the iodo compound **3**,[1] which was treated with DPA in the presence of K_2CO_3 in acetonitrile to afford DA **1**. After purification of DA **1** by recrystallization, it was treated with zinc perchlorate hexahydrate in acetonitrile to afford the desired **1**·Zn(II) complex.



Scheme 1. A new synthetic route to acedan and 1·Zn(II). Reagents and conditions: (a) $LiN(SiMe_3)_2$, I_2, THF, -78 to 0 °C (95%); (b) DPA, K_2CO_3, CH_3CN, 80 °C, 2 h (75%); (c) $Zn(ClO_4)_2.6H_2O$, CH_3CN, r.t, 10 h (95%)

Synthesis of 6-dimethylamino-2-(2'-iodoacetyl)-naphthalene (3).[1] Compound **3** was synthesized from acedan (**2**), according to the literature procedure. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.36 (1H, d, J = 1.8 Hz), 7.89 (1H, dd, J = 9.0 Hz), 7.79 (1H, d, J = 12.0 Hz), 7.62 (1H, d, J = 9.0 Hz),7.15 (1H, dd, J = 9.0 Hz), 6.86 (1H, d, J = 3.0 Hz), 4.44 (2H, s), 3.13(6H, s).

Synthesis of 2-(bis(pyridin-2-ylmethyl)amino)-1-(6-(dimethylamino)-naphthalen-2-yl)ethanone (DA 1). A solution of compound **3** (0.3 g, 0.89 mmol) in CH_3CN (20 mL) was treated with 2, 2′-dipicolylamine (153 µL, 0.85 mmol) and K_2CO_3 (123 mg, 0.89 mmol) under an argon atmosphere. The mixture was heated to reflux for 2 h, and then cooled to room temperature; it was diluted with CH_2Cl_2 (20 mL), the inorganic salts were filtered off, and the filtrate was concentrated. The residue was triturated with CH_2Cl_2 and hexane to remove the impurity as solid. The filtered solution was evaporated to

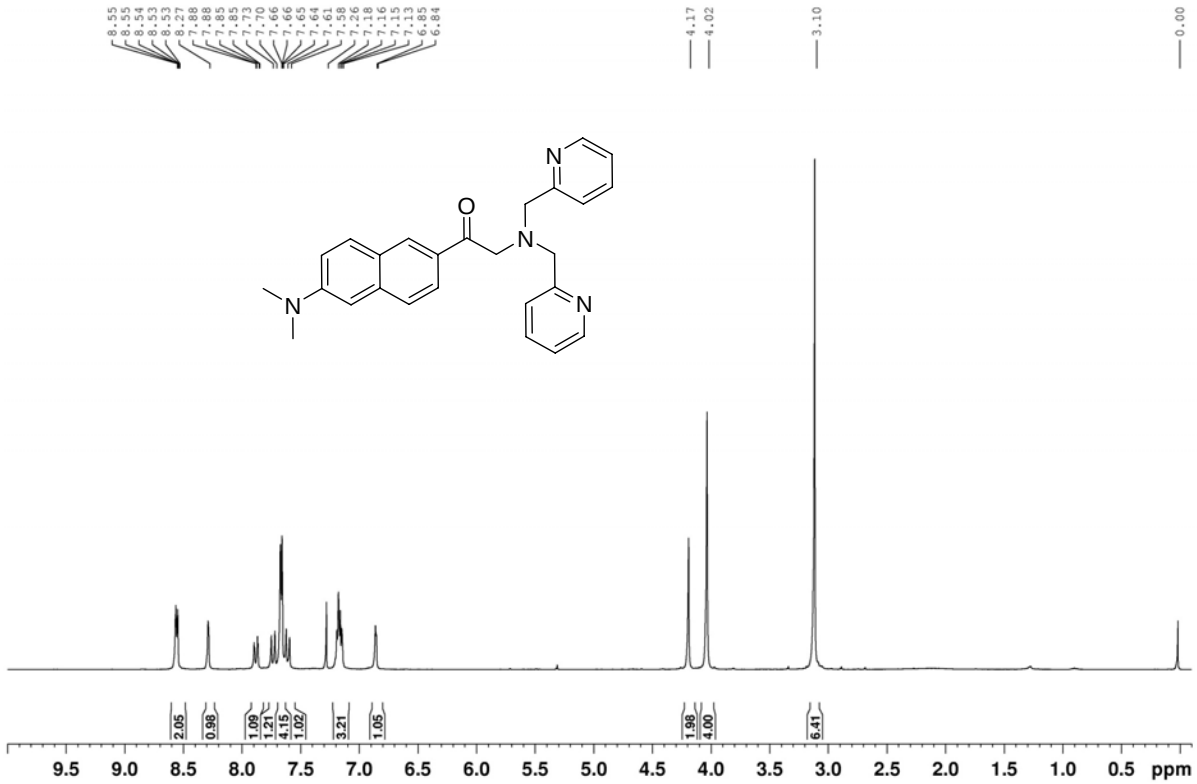
dryness and the residue was purified by crystallization from CH_2Cl_2 and hexane to afford DA **1** as a pale yellow solid (0.36 g, 75 %): mp 121–123 °C; IR (KBr, cm^{-1}) 1676, 1622; ^1H NMR (300 MHz, $CDCl_3$): δ 8.53 (2H, d, J = 3.0 Hz), 8.27 (1H, s), 7.85 (1H, dd, J = 8.7 Hz), 7.71 (1H, d, J = 9.0 Hz), 7.64 (4H, m), 7.58 (1H, d, J = 8.7 Hz), 7.13 (3H, m), 6.84 (1H, d, J = 2.1 Hz), 4.17 (2H, s), 4.01 (4H, s), 3.10 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.92, 159.36, 150.25, 148.87, 137.7, 136.59, 130.7, 129.94, 129.53, 126.07, 124.92, 124.42, 123.57, 122.12, 116.2, 105.28, 60.51, 59.75, 40.39.

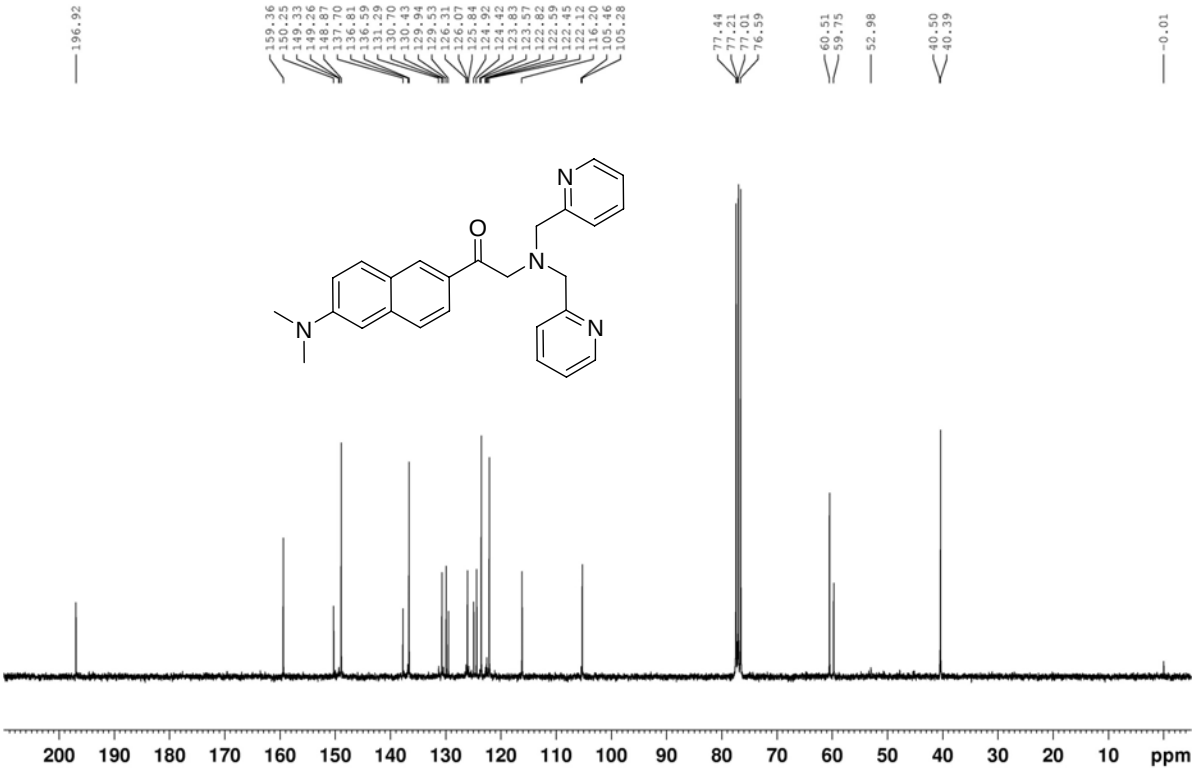
Synthesis of 1·Zn(II). A mixture of DA **1** (20 mg, 48.8 mmol) and zinc perchlorate hexahydrate (18.17 mg, 48.8 mmol) in CH_3CN was stirred at room temperature for 10 h. The reaction mixture was condensed by evaporation of the solvent under vacuum below 30 °C, and the residue, after trituration with CH_2Cl_2 and hexane, was filtered to give the zinc complex as a red solid (31 mg, 95 %): mp 207 °C (decompose); IR (KBr, cm^{-1}) 1610, 1577.7, 1502.5; ^1H NMR (300 MHz, CD_3CN): δ 8.73 (2H, d, J = 6.0 Hz), 8.42 (1H, s), 8.05 (2H, m), 7.82 (1H, d, J = 9.0 Hz), 7.71 (1H, dd, J = 9.0 Hz), 7.62 (3H, m), 7.56 (2H, m), 7.23 (1H, dd, J = 9.0 Hz), 6.91 (1H, s), 4.72 (2H, s), 4.37 (4H, m), 3.10 (6H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 198.27, 154.04, 151.03, 147.26, 140.59, 138.43, 131.99, 131.22, 126.14, 125.39, 124.88, 123.77, 123.56, 116.75, 104.54, 62.48, 60.96.

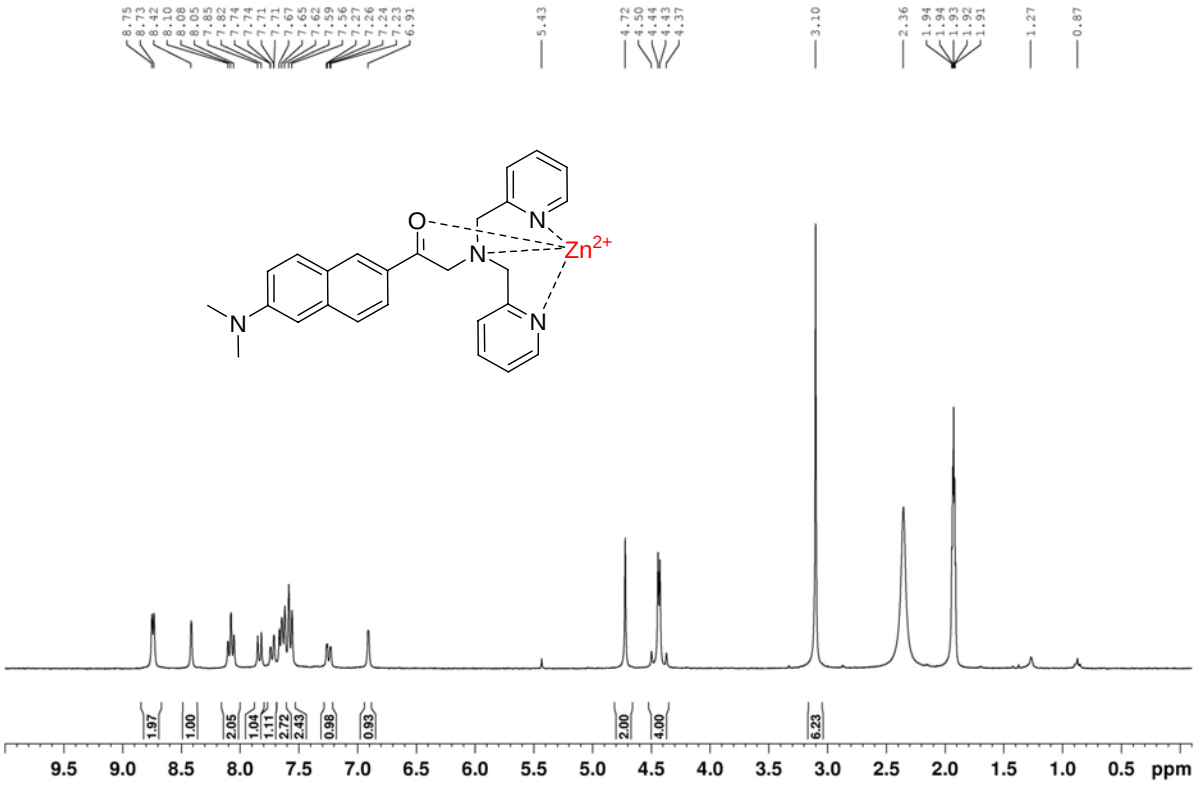
References

(1) (a) B. E. Cohen, T. B. Mc. Ananey, E. S. Park, Y. N. Jan, S. G. Boxer and L. Y. Jan, *Science* 2002, **296,** 1700. (b) C. H. Ru, G. X. Kun and Z. X. Bin, *Chinese J. Chem.* 2006, **24,** 1411.

^{1}H and ^{13}C NMR of DA 1 and 1·Zn(II)



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8.73
8.42
8.10
8.08
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7.85
7.82
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7.62
7.59
7.56
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7.23
6.91
5.43
4.72
4.50
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2.36
1.94
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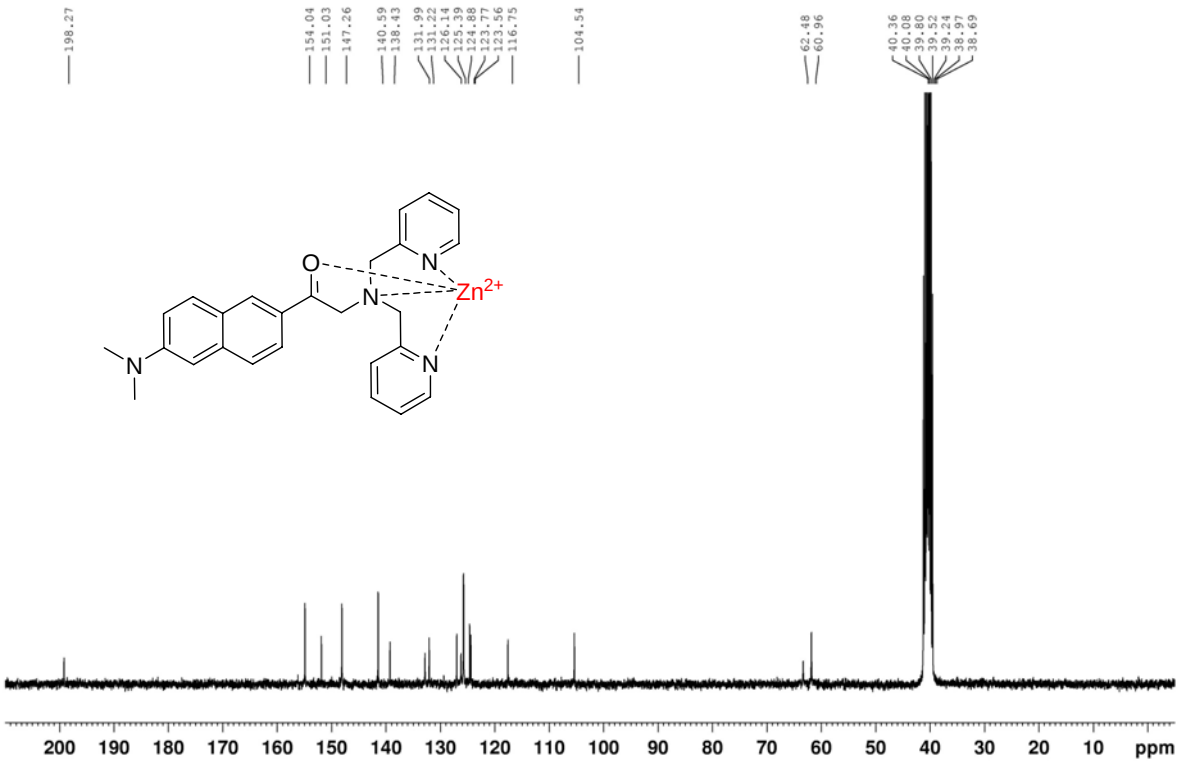
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154.04
151.03
147.26
140.59
138.43
131.99
131.22
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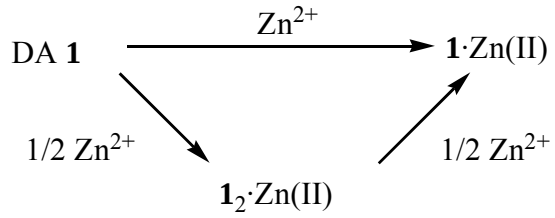
125.39
124.88
123.77
123.56
116.75
104.54
62.48
60.96
40.36
40.08
39.80
39.52
39.24
38.97
38.69
O
N
N
N
Zn2+
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Binding data between DA 1 and Zn^{2+}



Scheme S2 A binding process between DA **1** and Zn^{2+}.

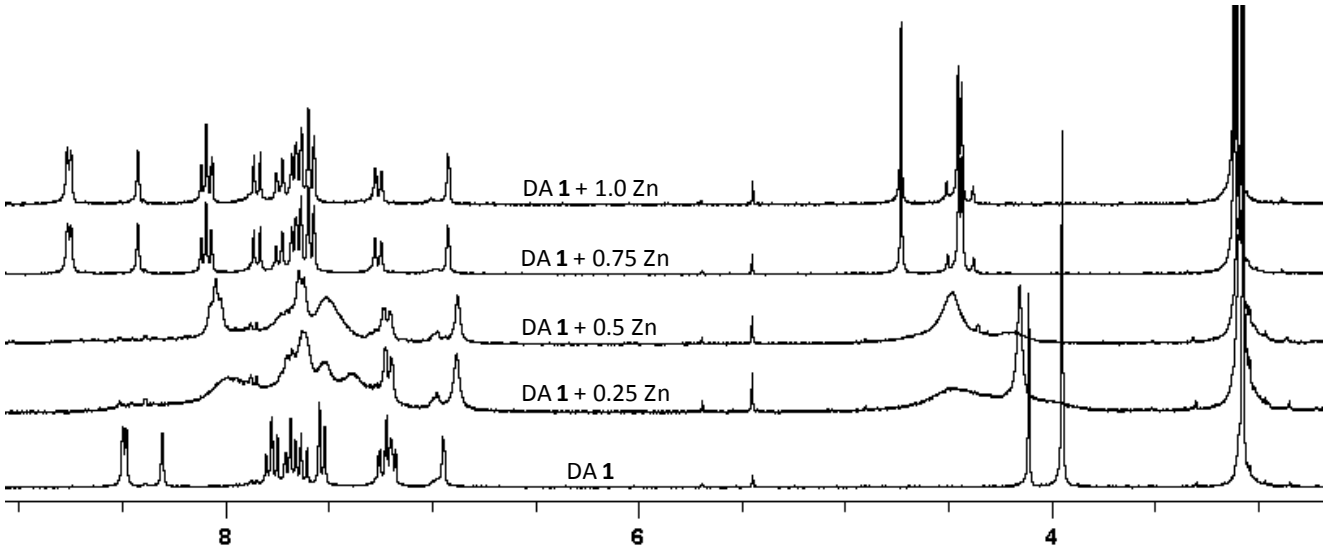


Fig. S1 ^{1}H NMR (300 MHz) spectra change of the DA **1** (10 mM) upon addition of Zn^{2+} (4 × 2.5 mM) in CD_3CN.

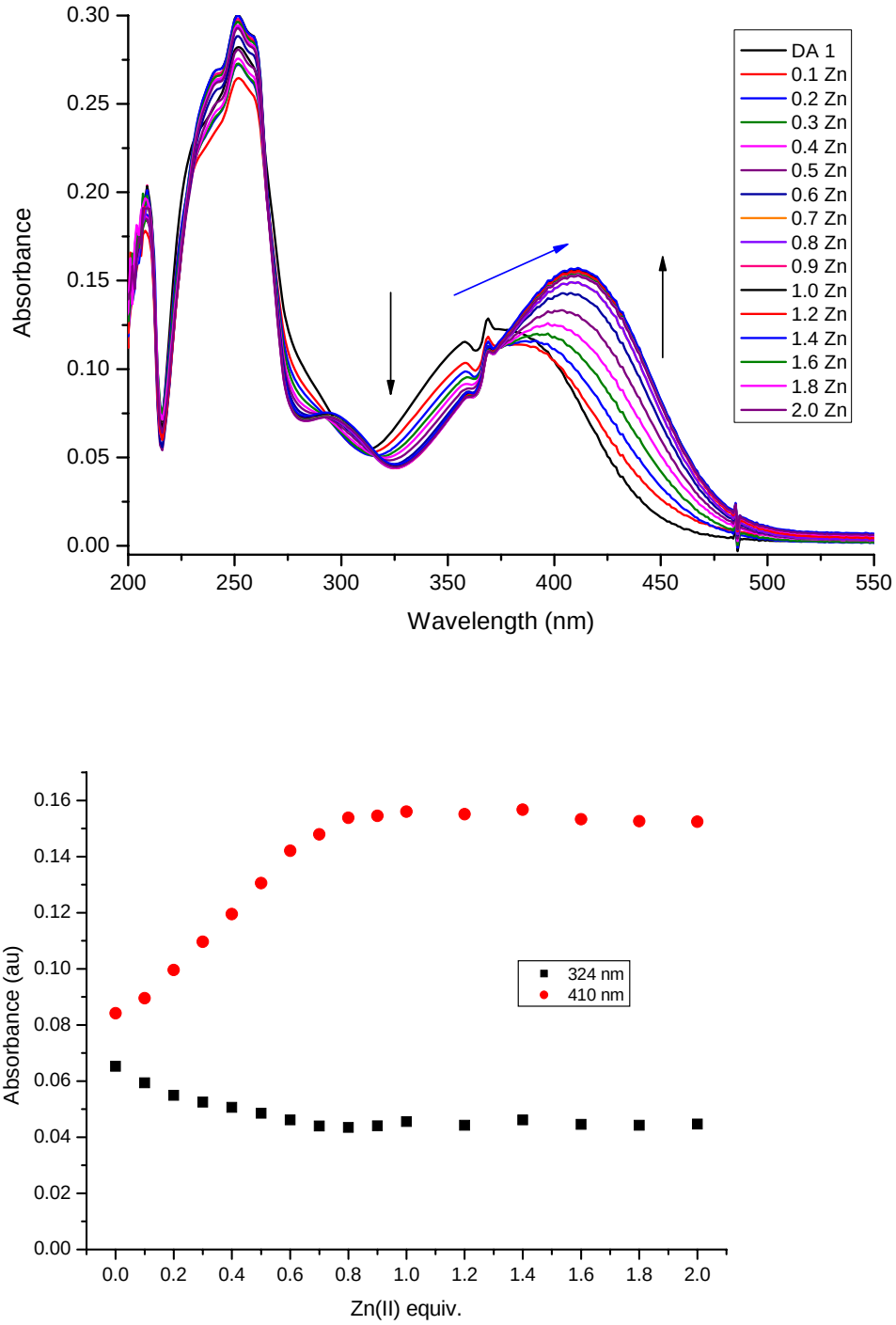
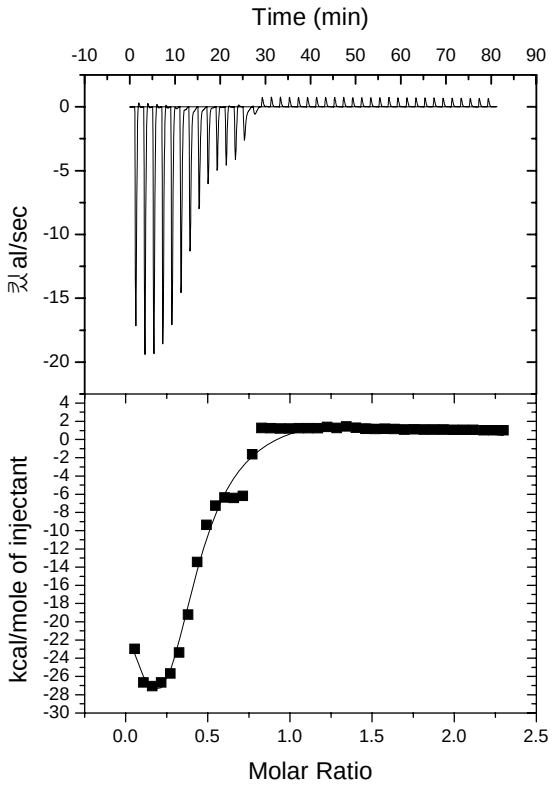


Fig. S2 UV absorption change of the DA **1** (10 μM) upon addition of $Zn(ClO_4)_2$ (0–20 μM) at 7.4 pH (10 mM HEPES containing 1% CH_3CN). Bottom: change in the absorbance intensity at 324 nm (decrease) and 410 nm (increase)

Isothermal Titration Calorimetry (ITC) Data



Data: dpalig1S1_NDH		
Model: Sequential Binding Sites		
Chi^2/DoF = 1.253E6		
K1	1.60E4	8.8E3
DH1	-2.881E4	5.80E3
DS1	-75.8	
K2	7.66E3	7.4E3
DH2	-1.384E5	2.71E5
DS2	-439	
K3	4.27E3	4.0E3
DH3	2.189E5	6.54E6
DS3	739	
K4	9.85E4	8.6E4
DH4	1.486E5	9.63E6
DS4	513	
K5	5.97E4	9.0E4
DH5	-4.889E4	4.99E6
DS5	-139	
K6	2.79E4	3.3E4
DH6	-2.998E5	2.00E6
DS6	-969	

Fig. S3 Isothermal titration calorimetry (ITC) data: (a) DA **1** (0.2 mM) with $Zn(ClO_4)_2$ (3.0 mM); measured in CH_3CN at 30 °C.

Procedure: To a solution of DA **1** in the calorimeter cell, 5.0 µL of zinc perchlorate was injected 40 times at 30 °C. The dilution effects were corrected by carrying out a separate blank titration. The titration data was analyzed by the built-in curve-fitting origin software.

Conditions:

Cell: DA **1** (0.2 mM) in acetonitrile

Syringe: Zinc perchlorate hexahydrate solution (3.0 mM) in acetonitrile

Reference power: 26; temperature: 30 °C; stirring rate: 220 rpm

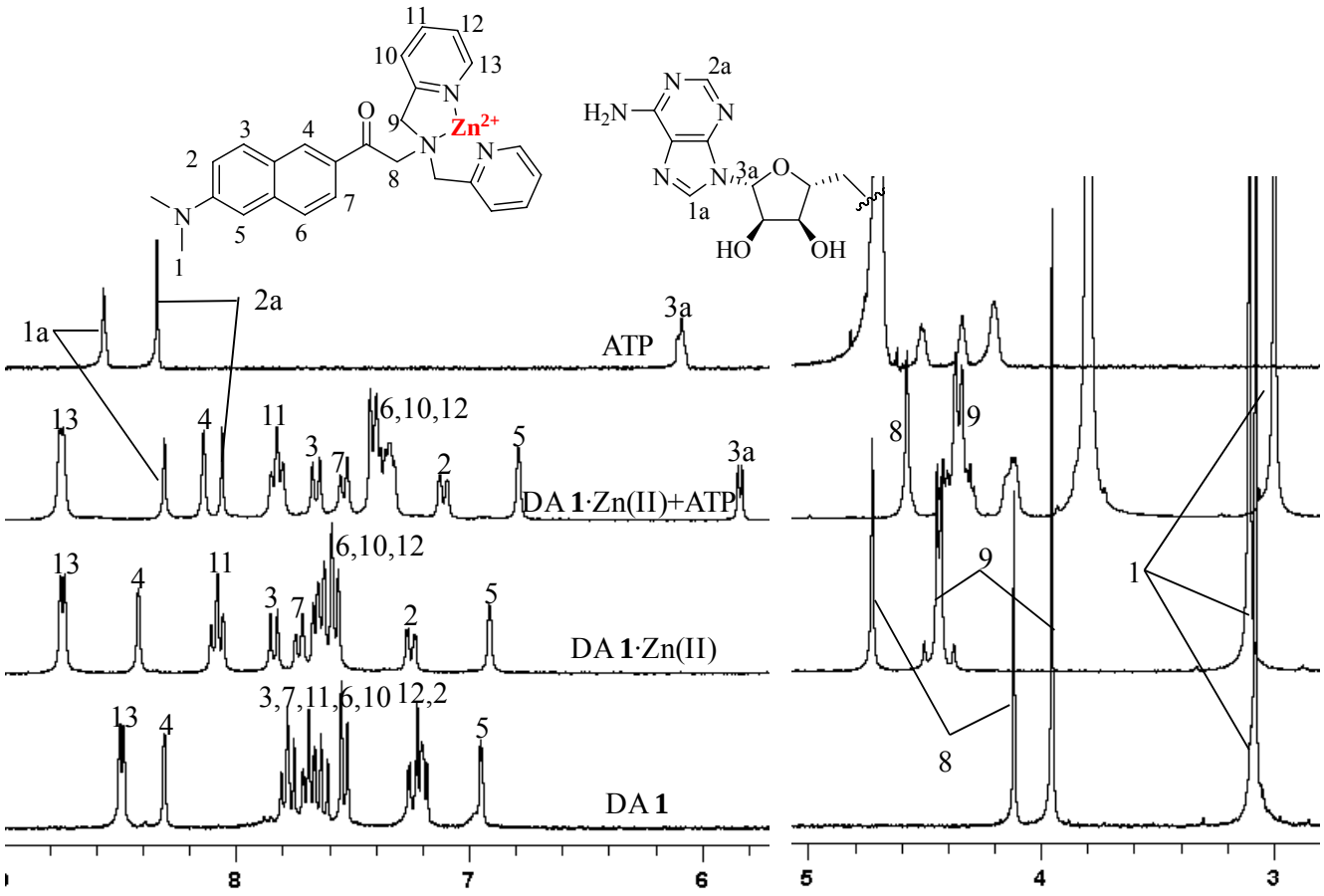


Fig. S4 ^{1}H NMR spectra for DA **1**, **1**·Zn(II) (10 mM), and **1**·Zn(II)·ATP (1:1:1 ratio) in CD_3CN, except for ATP that was taken in D_2O.

Infrared Spectroscopy (IR) data of DA 1 and 1·Zn(II)

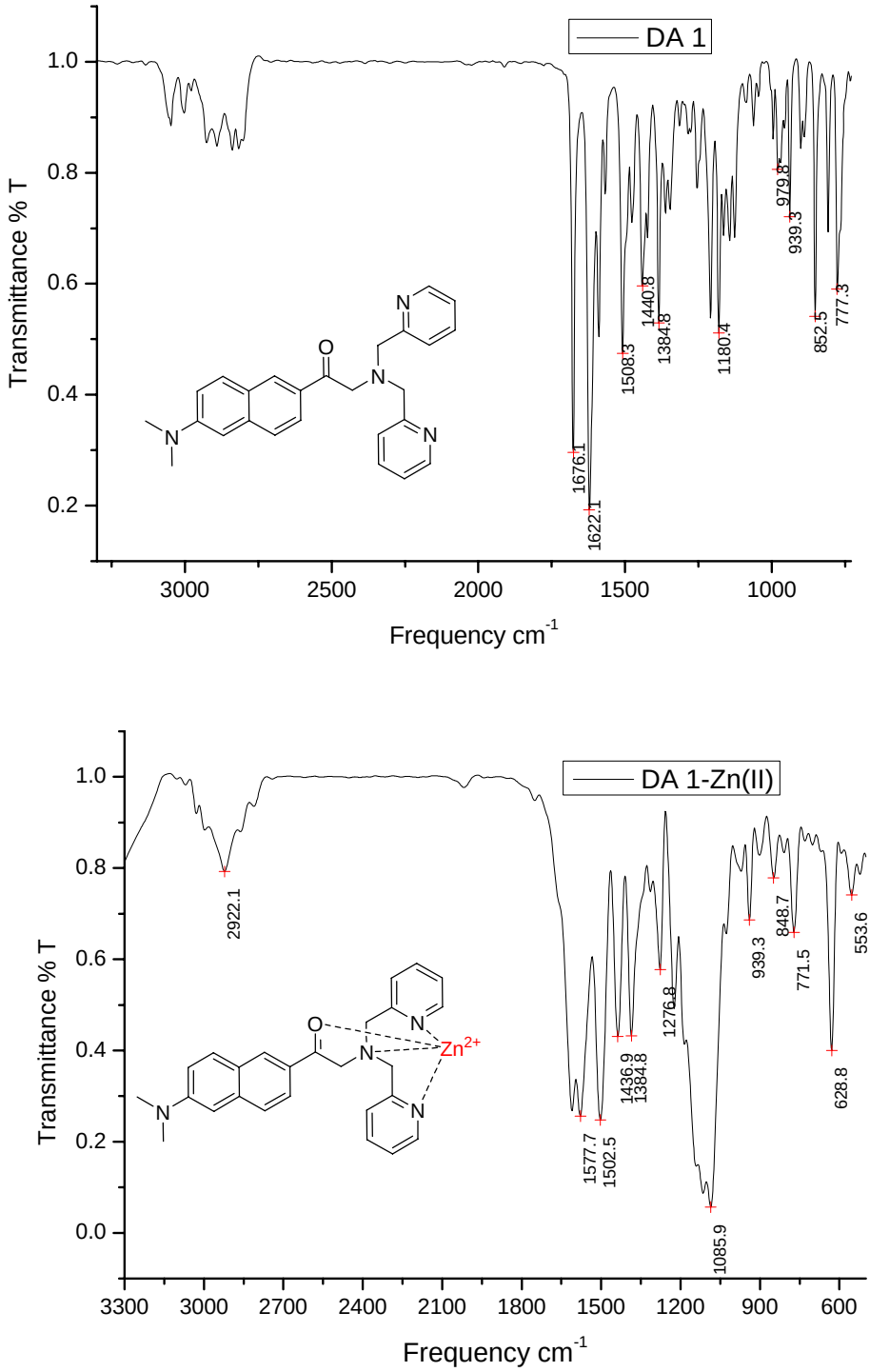


Fig. S5 Infrared Spectra of DA **1** (KBr) and **1**·Zn (II) (KBr)

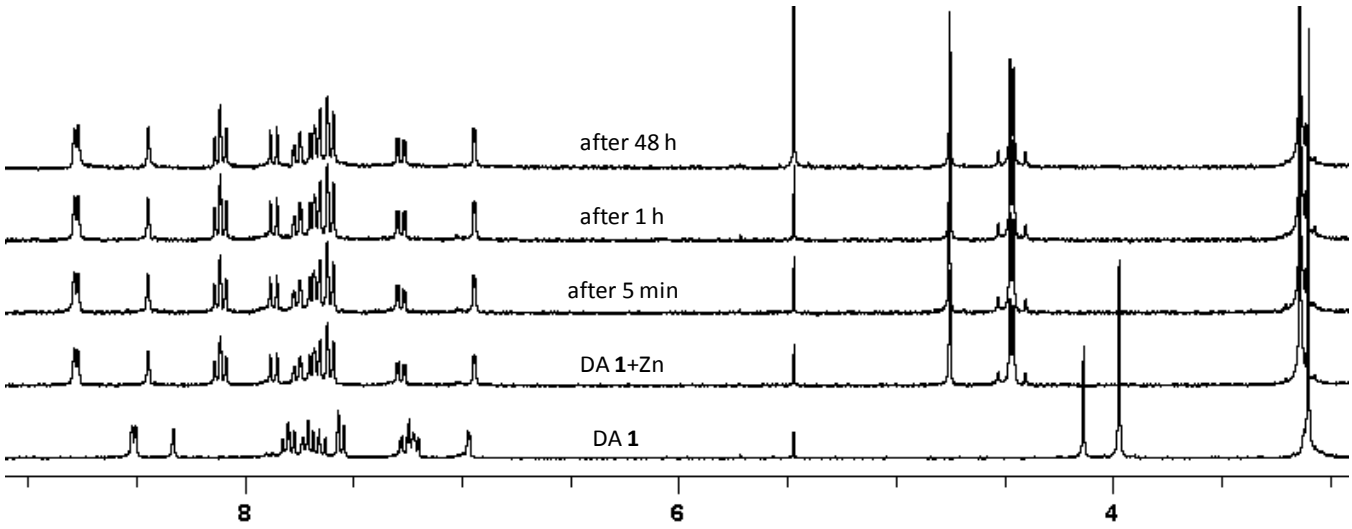


Fig. S6 Time-dependent ^{1}H NMR (300 MHz) spectral change of a 1:1 mixture of DA **1** and Zn^{2+} (1 equiv.) in CD_3CN.

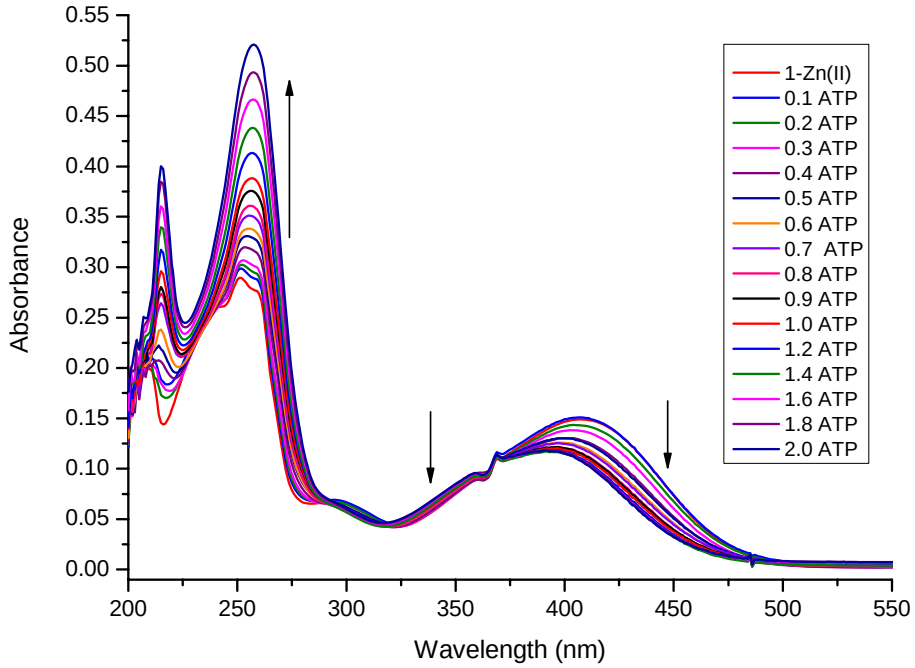


Fig. S7 UV absorption change of **1**·Zn(II) (10 μM) upon addition of ATP (0–20 μM) at 7.4 pH (10 mM HEPES buffer containing 1% CH_3CN).

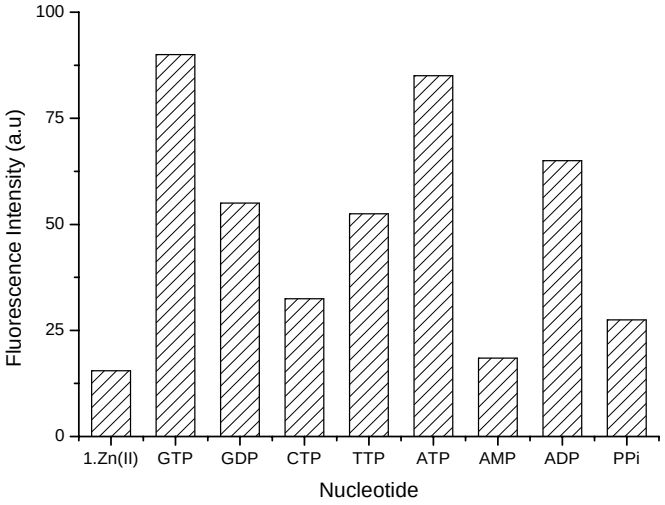


Fig. S8 Fluorescence spectral change of **1**·Zn(II) (10 μM) upon addition of sodium salt of GTP, GDP, CTP, TTP, ATP, AMP, ADP and PPi (1 equiv.) at 7.4 pH (10 mM HEPES containing 1% CH_3CN) (λ_{ex} = 370 nm).

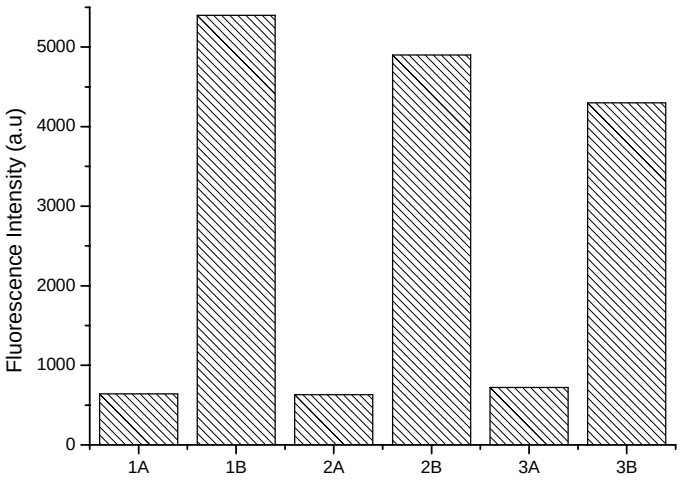


Fig. S9 Fluorescence spectral changes of **1**·Zn(II) (10 μM) upon addition of sodium salt of ATP (5 equiv.) at 7.4 pH (1% CH_3CN in three different buffers 1, 2 and 3) (λ_{ex} = 370 nm). A: **1**·Zn (10 μM) only; B: after addition of 5 equiv. ATP. Buffer 1: 10 mM HEPES (pH 7.4); Buffer 2 (HEPES-buffered saline): 107 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 11.5 mM glucose, 20 mM HEPES, pH 7.4; Buffer 3: HEPES-buffered saline supplemented with 3 mM glutathione, 3 mM inorganic phosphate, and 50 μM inorganic pyrophosphate in order to mimic cytosolic conditions.

Cell culture and Two-photon imaging

Cell culture. Seven cultured cells are: (1) A549 is a lung cancer cell; (2) MCF-7 is a breast cancer cell; (3) PANC-1 is a pancreatic carcinoma cancer cell; (4) PC3 is a prostate cancer cell; (5) PNT-2 is a prostate normal cell; (6) AsPC-1 is a pancreatic tumer cell; (7) SNU-216 is a gastric cancer cell.

Cell toxicity data. To evaluate cytotoxicity of the ATP probe **1**·Zn(II), Trypan blue assay performed for PC-3 cells. PC3 cell was cultured in the conditions of RPMI-1640 (Hyclone), which was supplemented with 10% (v/v) FBS (Hyclone), 100 U/mL penicillin G (Hyclone), and 100 mg/mL streptomycin (Hyclone) at 37 ºC in a 5% (v/v) CO_2 humid incubator. After culturing the cells on a circular dish, the cells were trypsinized and resuspended in serum-free RPMI-1640 media. Twenty μL of 2×10^4 cells were subcultured to 80% (v/v) occupation on a 24-well plate in the humid CO_2 incubator.

To verify the viability of the cell, various concentrations of the ATP probe (1, 10, 100, and 200 μM) were respectively treated to each well for diverse incubation time (6, 12, 24, 36, and 48 h). And then, cells on the each well were physically detached by pipetting and collected in the Eppendorf tube. Suspensions of each cell in the tube were combined with an equal volume of 0.1% (v/v) trypan blue solution for 5 min. Cells were placed in a hemocytometer, which counted the dead and viable cells. Viability could then be calculated using the following equation;

$$\% \text{ viability} = \frac{\text{live cells}}{(\text{dead} + \text{live cells})} \text{ X } 100$$

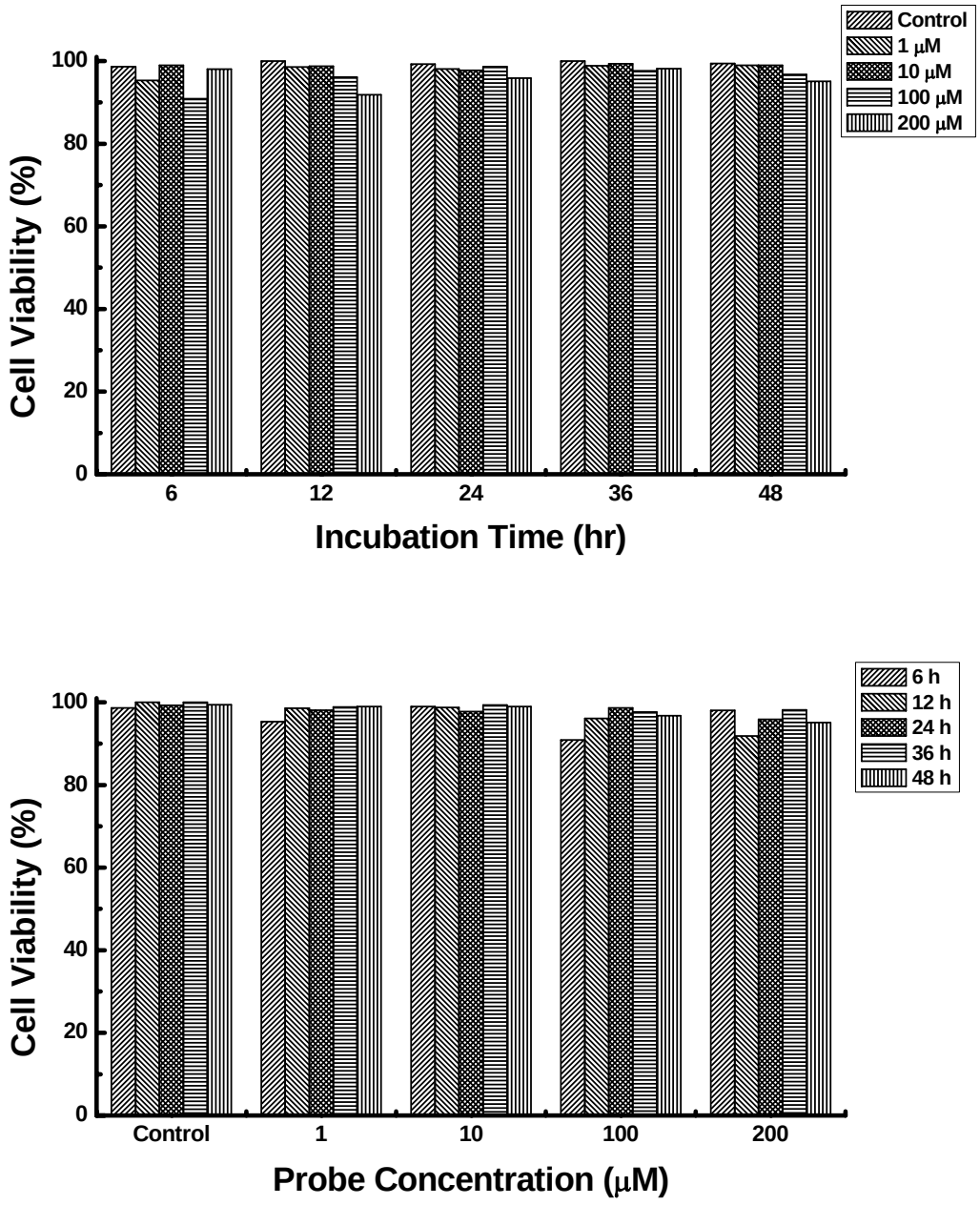


Fig. S10 Cytotoxicity data of PC3 cells by trypan blue assay, with 1, 10, 100 and 200 μM concentration of **1**·Zn(II) for 6, 12, 24, 36 and 48 h.

Preparation of cell lines for imaging: Seven cell lines were prepared for *in vivo* measurement. Two groups of cells were cultured in the conditions of RPMI-1640 (Hyclone), which was supplemented with 10% (v/v) FBS (Hyclone), 100 U/mL penicillin G (Hyclone), and 100 mg/mL streptomycin (Hyclone) at 37 ºC in a 5% (v/v) CO_2 humid incubator. After culturing the cell lines on a circular dish, the cells

were trypsinized and resuspended in serum-free RPMI-1640 media. Two hundred μL of 2×10^5 cells were sub-cultured to 80% (v/v) occupation on a 35 Π dish in the humid CO_2 incubator.

To confirm the effect of the probe, one group of cells were exposed to 100 μM ATP probe, **1**·Zn(II) for 5 min , and then each cell was washed with PBS buffer more than three times. The cells were used in 2 mL of PBS buffer for the further fluorescent imaging.

One-Photon Fluorescence Microscopy. The Axiovert 200M fluorescence imaging microscope (Carl Zeiss Co.) was used to capture fluorescent images using excitation (480-500 nm) and dichromatic emission (530-550 nm) filters.

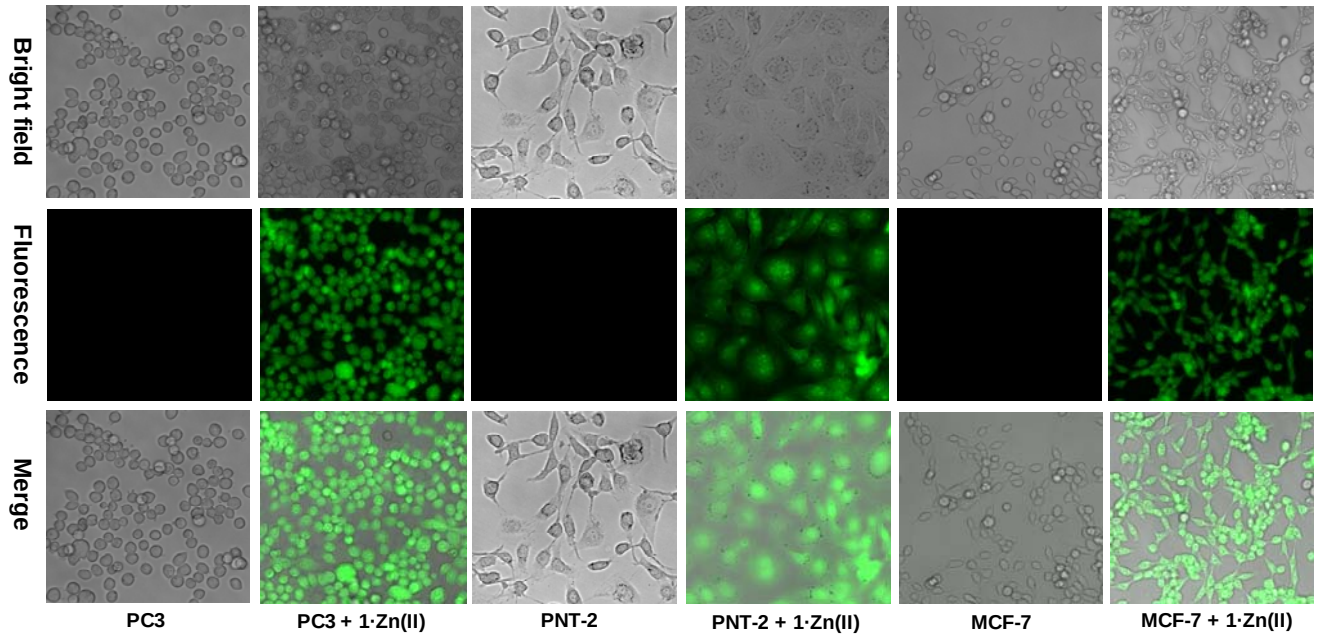


Fig. S11 One-photon bright field, fluorescence and merge images of the intracellular ATP stores of PC3, PNT-2 and MCF-7 cells, before and after treatment with **1**·Zn(II) (100 μM) for 5 min.

Two-Photon Fluorescence Microscopy. Two-photon fluorescence microscopy images of **1**·Zn(II) labeled cells were obtained with an upright microscope (BX51; Olympus) to build a point scanning two-photon microscopy. This system is equipped with Ti:sapphire femtosecond laser (Chameleon; Coherent) with a tuning range of 680 to 1020 nm and we used 740 nm for imaging. To generate two-photon effect, laser source has 80 MHz repetition rate and 140 fs pulse width. It first passed through a half wave plate

and a polarizer for power control and it is expanded 2times by passing a pair of plano-convex (PCX) lens, and then expanded excitation light pass through the x-y scanner. We used resonant scanner (counter rotation scanner, GSI Lumonics) which has about 8 KHz scanning speed and galvanometer mirror (6215H; Cambridge Technology) for y-axis scanning to perform high-speed scanning system. We used a pair of lens to conjugate scanner and back-aperture of objective, and focal length of each lens is 50 mm and 250 mm for utilizing enough numerical aperture (NA) of objective. After passing the scanner, excitation light go through the 680 nm short pass dichroic mirror (680dcspxr; Chroma) and the 680 nm long pass dichroic mirror (680dcxxr, Chroma) and then enter the objective. The scanned excitation light was focused onto the specimen through the objective (XLUMPLFLN-W 20X/1.0 water immersion; Olympus). Focused excitation light caused two-photon effect in the specimen and it emits the fluorescence. Emitted fluorescence goes back to the objective and reflects to the detection path by 680 nm long pass dichroic mirror. Reflected fluorescence pass through a pair of lens to improve efficiency of acquisition, and we used 2-inch dichroic mirror to gather more fluorescence by scattering for improving imaging depth. After that, emission fluorescence was transmitted and reflected by 570 nm long pass dichroic (570dc, Chroma). Each of them was collected by each photomultiplier tubes (PMTs, R5929, Hamamatsu) to generate two-color image. Signals from the PMTs were processed by a frame grabber (Alta, Bitflow) and two-color images were displayed in real time. The piezo stage (P725; PI) which can control 0 to 400 μm length is used for imaging the depth direction.