Supporting Information for

A novel platform self-assembled from squaraine-embedded Zn(II) complexes for selective monitoring of ATP and its level fluctuation in mitotic cells

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Materials

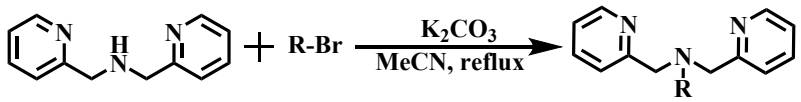
Unless otherwise stated, all chemicals and reagents were obtained from commercial suppliers and used without further purification. Ethanol, methanol, acetonitrile and dichloromethane were purchased from KeLong (Chengdu, China), while 1-bromododecane, 1-bromohexadecane and zinc chloride were purchased for Aladdin Industrial Inc (Shanghai, China). MeOH and MeCN were purified and redistilled by standard methods prior to use. Water used was deionized. Gastric cancer SGC-7901 cells were purchased from Shanghai Institute of Materia Medica, Chinese Academy of Science. Cationic squaraine dye (**SQ**)[S1] and bis(2-pyridylmethyl)amine (DPA)[S2] were synthesized and purified as literatures reported previously.

Measurements

^{1}H NMR and ^{13}C NMR were collected on a Bruker 500 avance III spectrometer. Mass spectrometric (MS) data were obtained with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. Absorption and emission spectra were collected by using

a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan), respectively. SEM images were observed with a JSM-6701F scanning electron microscope. Surface tension wes collected by using a BZY-3B automatic surface tensiometer. Diameter distribution was obtained with Delsa Nano C analyzer (Beckman Coulter, Inc).

Synthesis of DPA-12



DPA

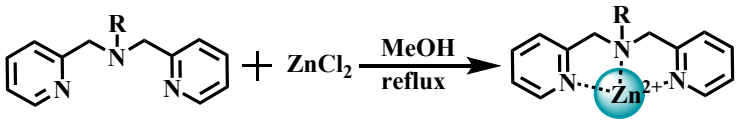
DPA-12 R=$C_{12}H_{25}$
DPA-16 R=$C_{16}H_{33}$

The mixture of di-(2-picolyl)amine (1.00 g, 5.02 mmol), 1-bromododecane (1.25 g, 5.02 mmol) and K_2CO_3 (2.08 g, 15.06 mmol) in acetonitrile was heated at reflux and stirred for 24 h. The mixture was allowed to cool and evaporated to dryness under reduced pressure. The crude product was purified on a flash column (CH_2Cl_2). The product was faint yellow oil, yield 78%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (d, J = 4.5 Hz, 2H), 7.51 (td, J = 7.7, 1.4 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 7.03 – 6.97 (m, 2H), 3.70 (s, 4H), 2.47 – 2.40 (m, 2H), 1.46 – 1.40 (m, 2H), 1.12 (d, J = 10 Hz, 18H), 0.76 (t, J = 6.8 Hz, 3H). HRMS found 368.3058 $(M+H)^+$, cald for ($C_{24}H_{38}N_3$) 368.2987.

Synthesis of DPA-16

The mixture of di-(2-picolyl)amine (1.00 g, 5.02 mmol), 1-bromohexadecane (1.53 g, 5.02 mmol) and K_2CO_3 (2.08 g, 15.06 mmol) in acetonitrile was heated at reflux and stirred for 24 h. The mixture was allowed to cool and evaporated to dryness under reduced pressure. The crude product was purified on a flash column (CH_2Cl_2). The product was faint yellow solid, yield 73%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, J = 4.2 Hz, 2H), 7.58 (td, J = 7.7, 1.7 Hz, 2H), 7.50 (d, J = 7.8 Hz, 2H), 7.10 – 7.03 (m, 2H), 3.78 (s, 4H), 2.54 – 2.46 (m, 2H), 1.50 (m, 2H), 1.23 (m, 26H), 0.83 (t, J = 6.9

Hz, 3H). MS found 424.67 $(M+H)^+$, cald for ($C_{28}H_{46}N_3$) 424.68.



DPA-12 R=$C_{12}H_{25}$
DPA-16 R=$C_{16}H_{33}$

DPA-12@Zn(II) R=$C_{12}H_{25}$
DPA-16@Zn(II) R=$C_{16}H_{33}$

Preparation of DPA-12@Zn(II)

The reaction mixture of **DPA-12** (0.50 g, 1.36 mmol) and $ZnCl_2$ (0.19 g, 1.36 mmol) in MeOH was refluxed for 3 h. After the reaction was completed, the mixture was evaporated to dryness under reduced pressure. The product was faint yellow solid and used for next test without any purification.

Preparation of DPA-16@Zn(II)

The reaction mixture of **DPA-16** (0.50 g, 1.18 mmol) and $ZnCl_2$ (0.16 g, 1.18 mmol) in MeOH was refluxed for 3 h. After the reaction was completed, the mixture was evaporated to dryness under reduced pressure. The product was faint yellow solid and used for next test without any purification.

Sample preparation and titration

DPA-12@Zn(II) (50 mM) and **DPA-16@Zn(II)** (50 mM) were obtained in MeOH as stock solution. Stock solution of **SQ** (0.5 mM) was prepared in EtOH and further diluted to 5.0 μM for titration experiments. Stock solutions of ATP, ADP, AMP, GTP, CTP, UTP, dTTP, Pi and metal ions were prepared in deionized water, and the concentrations are 10 mM. The samples for SEM experiments were obtained by applying a drop of solution with micelles (200 μM of **DPA-16@Zn(II)** with 5 μM of **SQ** or 400 μM of **DPA-12@Zn(II)** with 5 μM) onto a silicon wafer followed by drying at room temperature.

Cell culture and fluorescence image

SGC-7901 cells were seeded in 35 mm glass-bottomed dishes (NEST) and incubated in Dulbecco's Modified Eagle medium (DMEM) media for 24 h. Before incubation with **SQ**-embedded **DPA-12@Zn(II)** micelles, the SGC-7901 cells were incubated with or without 5 mM of Ca^{2+} for 30 min. Then, 1 mL of fresh cell medium with the **SQ**-embedded **DPA-12@Zn(II)** micelles ([**SQ**]=5.0 μM, [**DPA-12@Zn(II)**]=400 μM) was added in each well, respectively. After incubation for 6 h, the cells untreated with Ca^{2+} were further incubated in fresh DMEM media containing of ATP (20 μM) for 30 min, followed by incubating with 5 μM of rhodamine 123. After incubation for 30 min, the cells were washed with phosphate buffered three times. Confocal fluorescence imaging of SGC-7901 cells was observed under an A1R Laser Scanning Confocal Microscope (Japan).

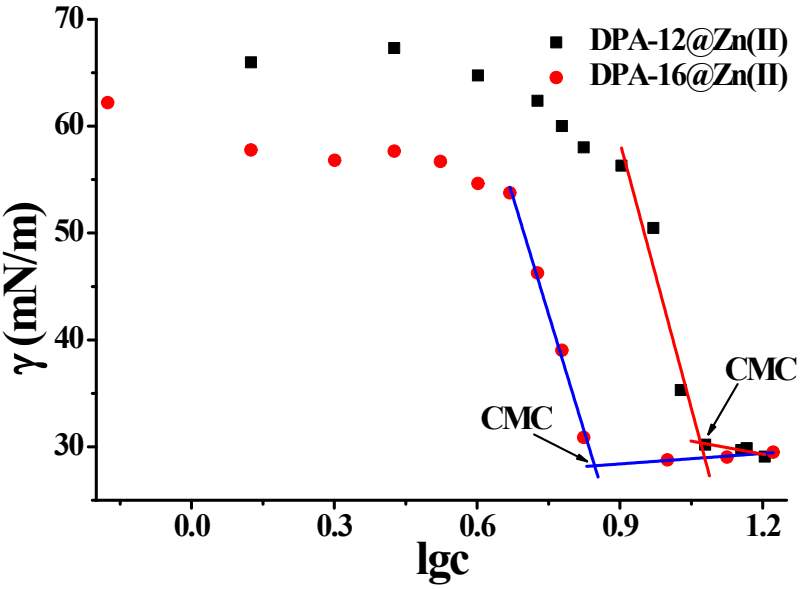


Fig. S1 The surface tension of **SQ** solution (5.0 μM) prepared by adding varied concentrations of **DPA-12@Zn(II)** (red line) and **DPA-16@Zn(II)** (black line) in phosphate buffer (10 mM, pH 7.2), respectively, T=298 K.

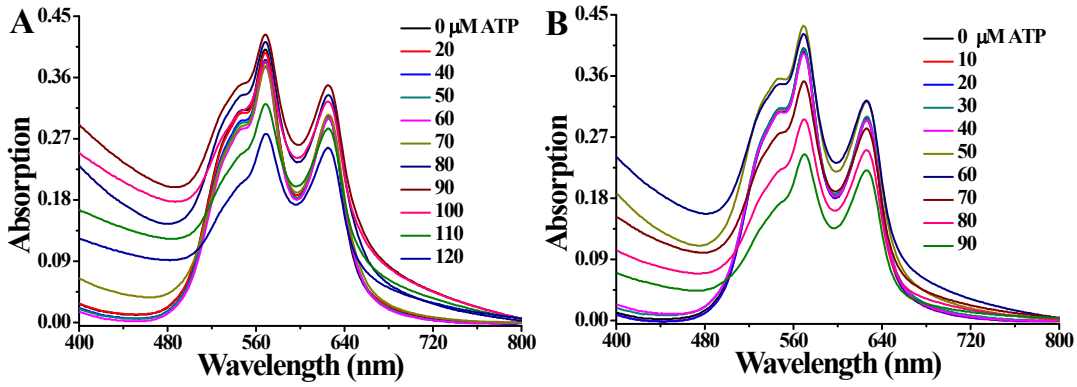


Fig. S2 UV-Vis absorption spectral changes of **SQ**-embedded self-assembly micelles (400 μM of **DPA-12@Zn(II)** with 5 μM of **SQ** (A) and 200 μM of **DPA-16@Zn(II)** with 5 μM of **SQ**) (B)) upon addition of ATP in phosphate buffer (10 mM, pH 7.2), respectively.

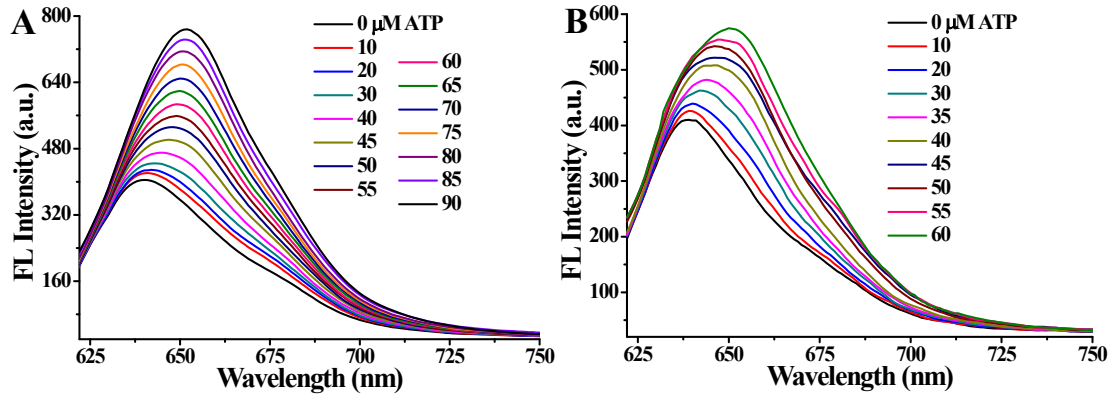


Fig. S3 Fluorescence spectra change of **SQ**-embedded micelles (400 μM of **DPA-12@Zn(II)** with 5 μM of **SQ** (A) and 200 μM of **DPA-16@Zn(II)** with 5 μM of **SQ**) (B)) upon addition of ATP in phosphate buffer (10 mM, pH 7.2).

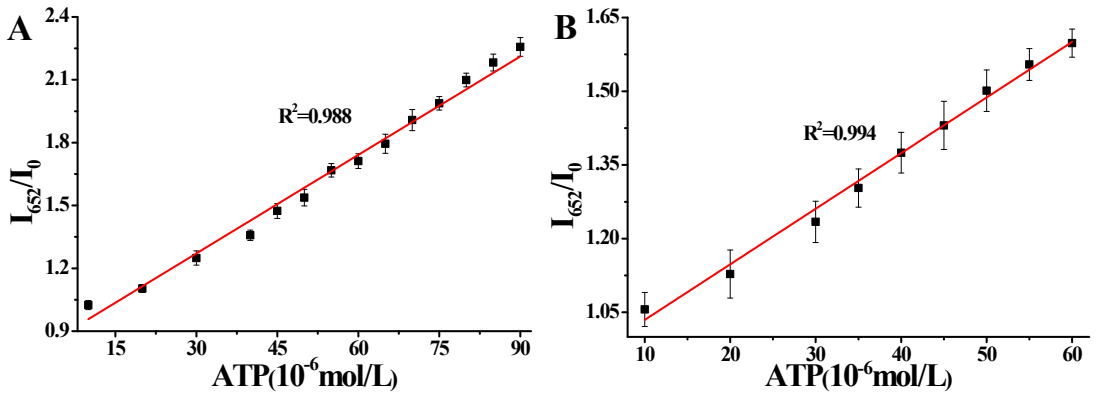


Fig. S4 The relative fluorescence intensity (I_{652}/I_0) of **SQ**-embedded self-assembly micelles (400 μM of **DPA-12@Zn(II)** with 5 μM of **SQ** (A) and 200 μM of **DPA-16@Zn(II)** with 5 μM of **SQ**) (B)) response to different concentration of ATP in phosphate buffer (10 mM, pH 7.2), respectively.

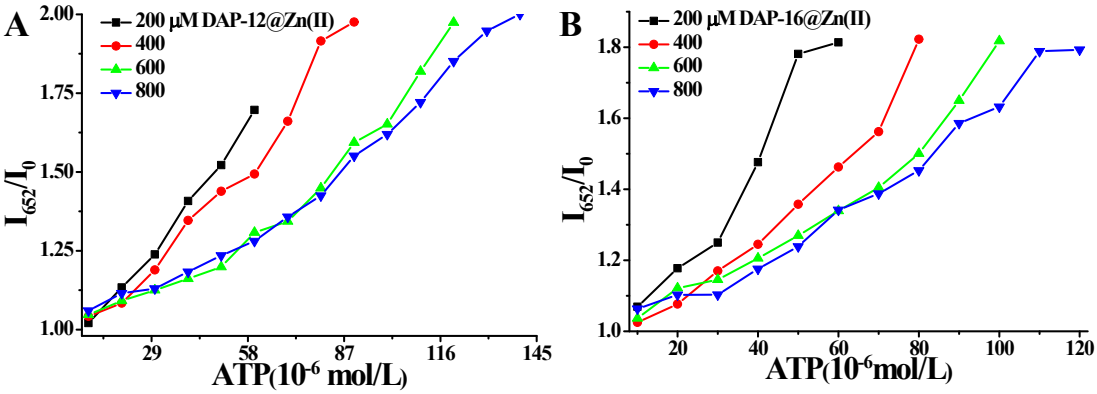


Fig. S5 The relative fluorescence intensity of **SQ** (5.0 μM) with different concentration of **DPA-12@Zn(II)** (A) and **DPA-16@Zn(II)** (B) in phosphate buffer (10 mM, pH 7.2) by addition of ATP. The concentrations of DPA-Zn(II) complex derivatives are 200, 400, 600, 800 μM, respectively.

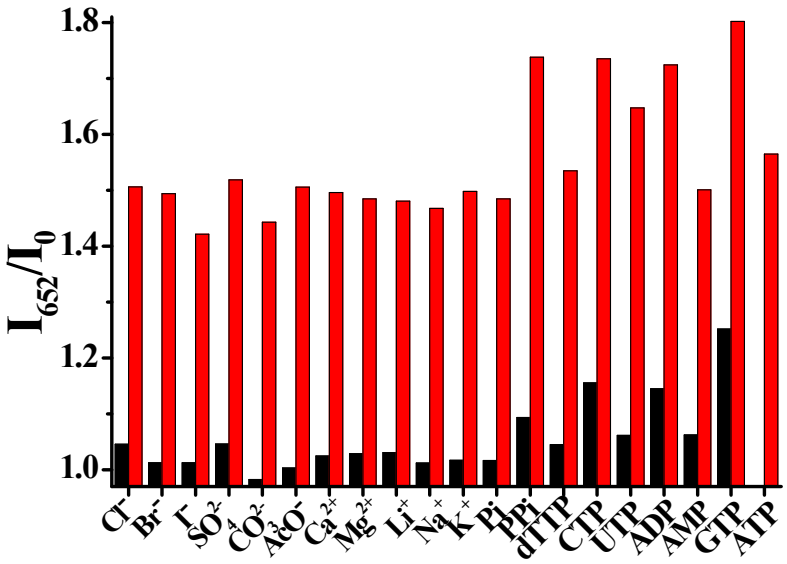


Fig. S6 The relative fluorescence intensity (I_{652}/I_0) of **SQ**-embedded self-assembly micelles (400 μM of **DPA-12@Zn(II)** with 5 μM of **SQ**) at 652 nm upon addition of various ions or other nucleoside polyphosphates (60 μM, black bars). Red bars represent the relative intensity with subsequent addition of ATP (60 μM).

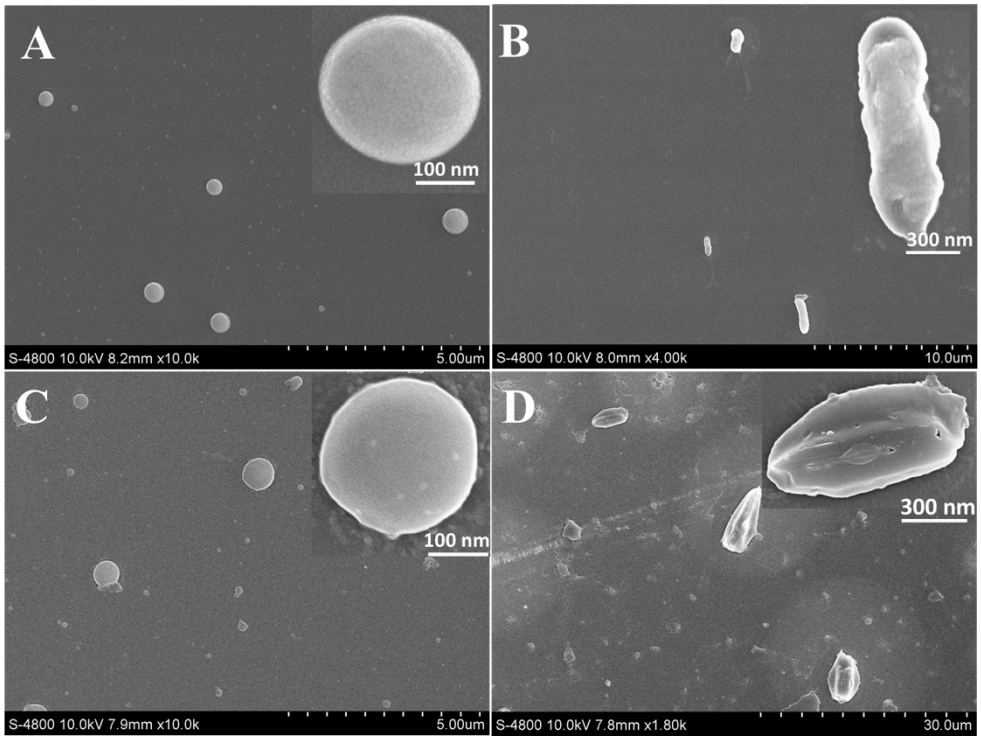


Fig. S7 SEM images of **SQ**-embedded **DPA-12@Zn(II)** and **DPA-16@Zn(II)** micelles before (A and C) and after (B and D) addition of ATP.

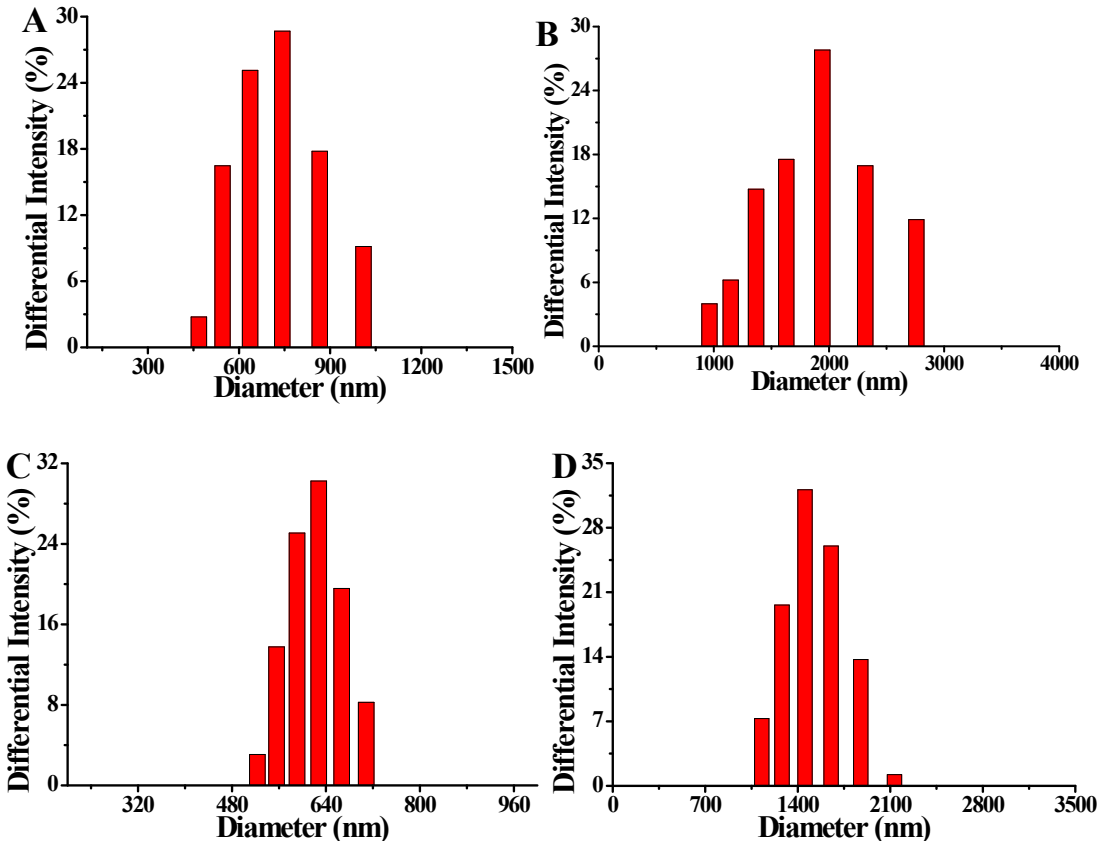


Fig. S8 DLS analysis of **SQ** (5 μM) in the presence of **DPA-12@Zn(II)** (400 μM) before (A) and after (B) addition of ATP (90 μM); DLS analysis of **SQ** (5 μM) in the presence of **DPA-16@Zn(II)** (200 μM) before (A) and after (B) addition of ATP (60 μM).

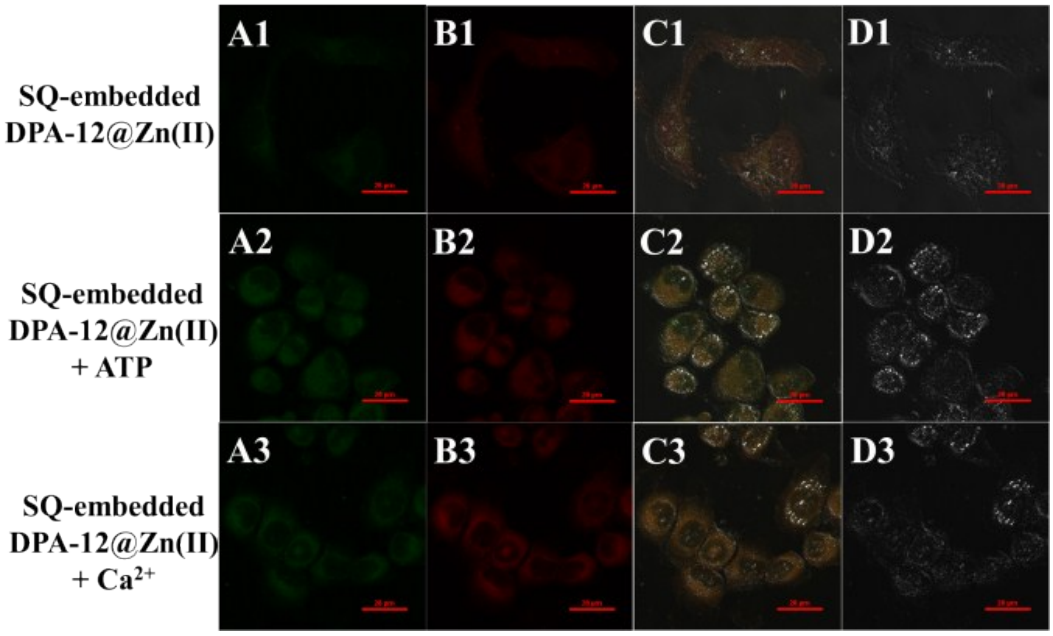


Fig. S9 Confocal fluorescence images of gastric cancer SGC-7901 cells incubated with **SQ**-embedded **DPA-12@Zn(II)** micelles after treatment for 30 min at 37ºC with (A3-D3) or without (A1-D1and A2-D2) Ca^{2+} (5 mM) in DMEM and then incubated with 20 μM of ATP (A2-D2) and Rhodamine 123 (5 μM). A) Green channel for Rhodamine 123. B) Red channel for **SQ**-embedded **DPA-12@Zn(II)** micelles ([**SQ**]=5.0 μM, [**DPA-12@Zn(II)**]=400 μM). C) The overlap of red channel, green channel and optical image. D) Optical image. Scare bar: 20 μm.

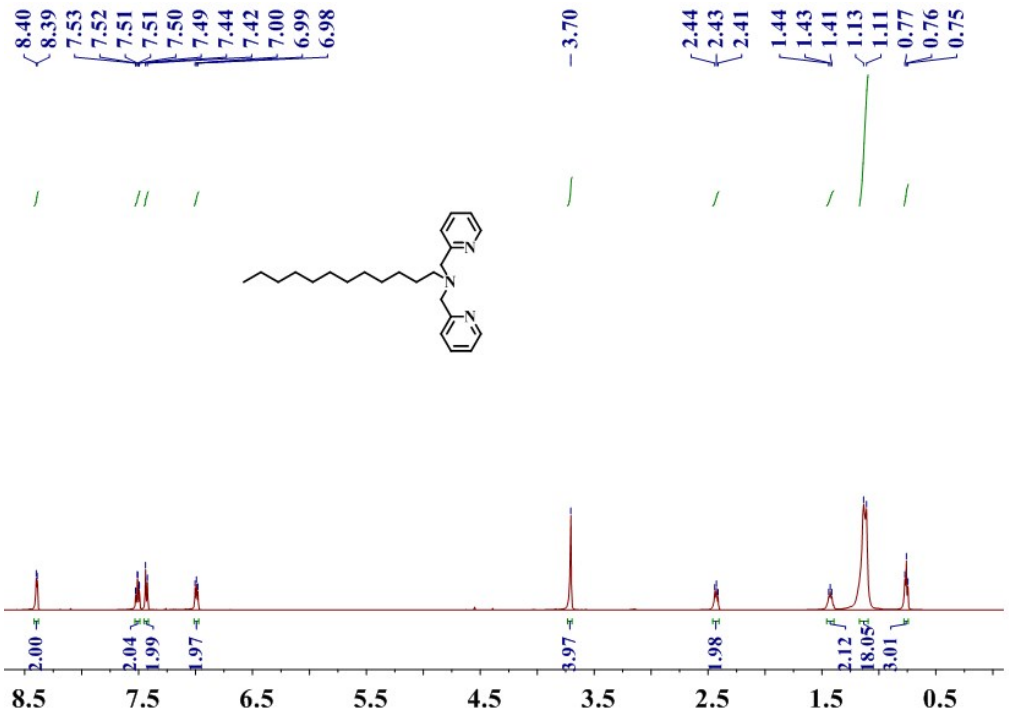


Fig. S10 ^{1}H-NMR Spectrum of compound **DPA-12** in $CDCl_3$.

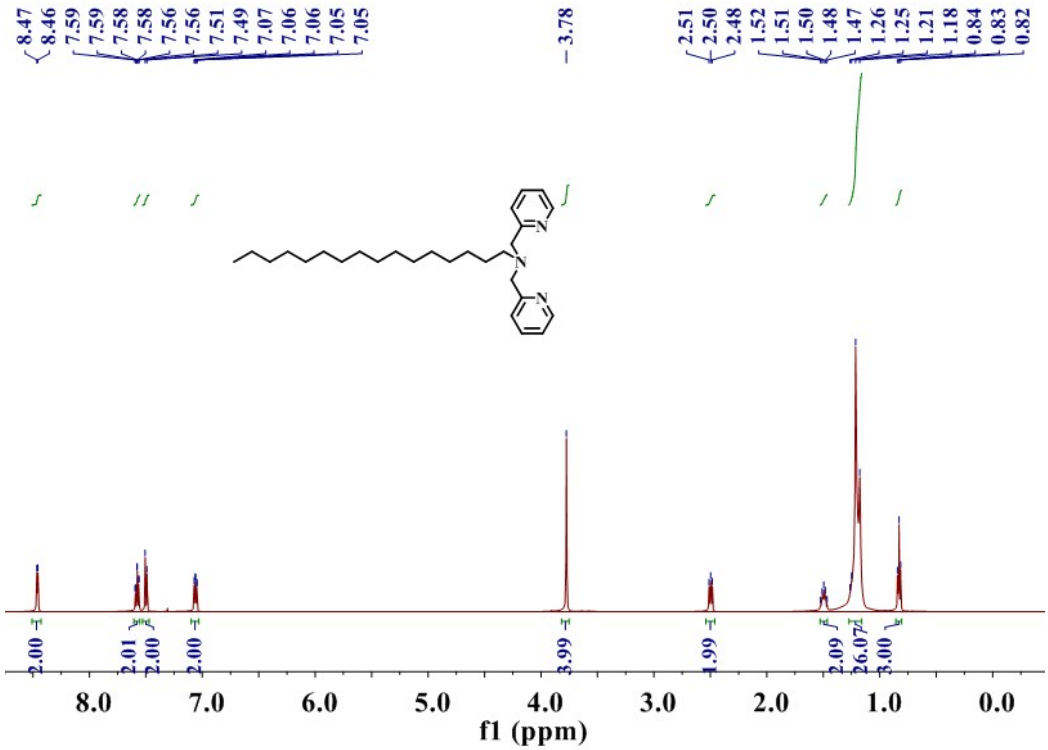


Fig. S11 ^{1}H-NMR Spectrum of compound **DPA-16** in $CDCl_3$.

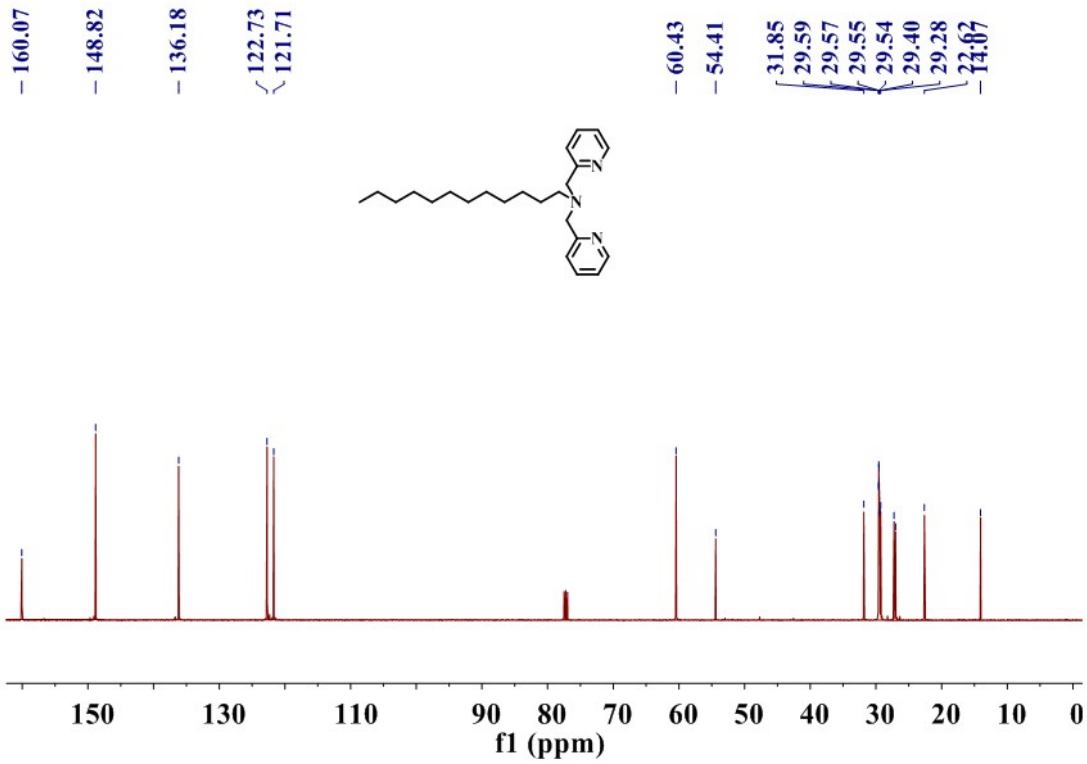


Fig. S12 ^{13}C-NMR Spectrum of compound **DPA-12** in $CDCl_3$.

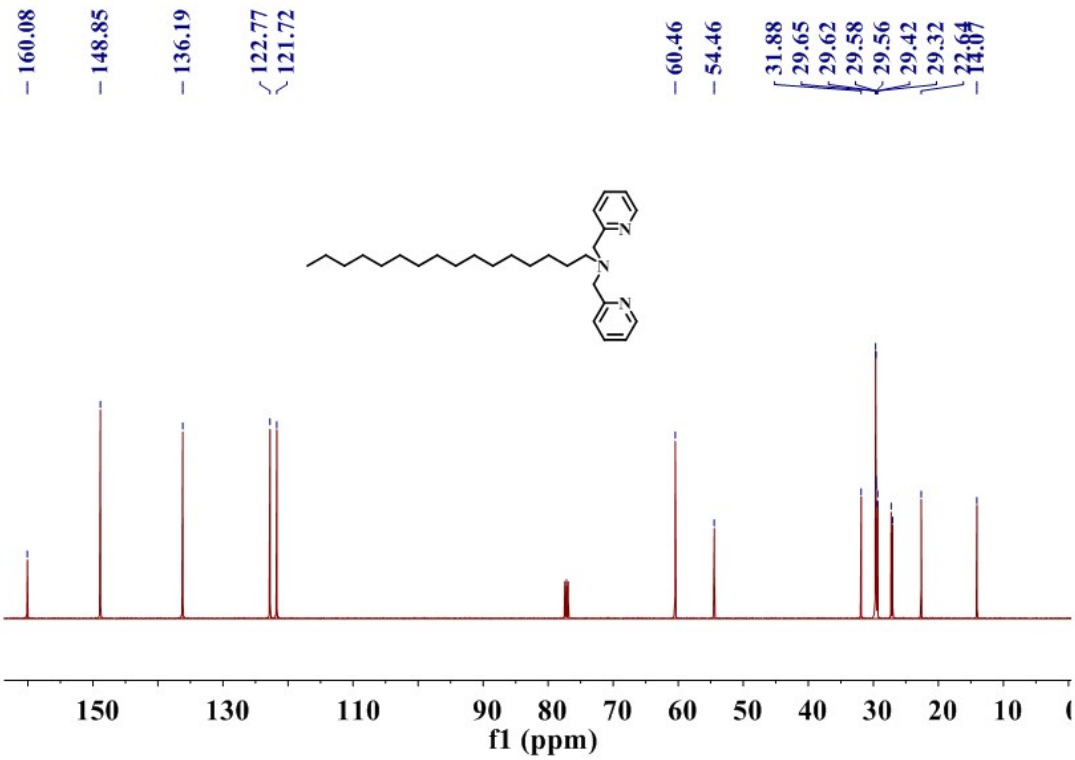


Fig. S13 ^{13}C-NMR Spectrum of compound **DPA-16** in $CDCl_3$.

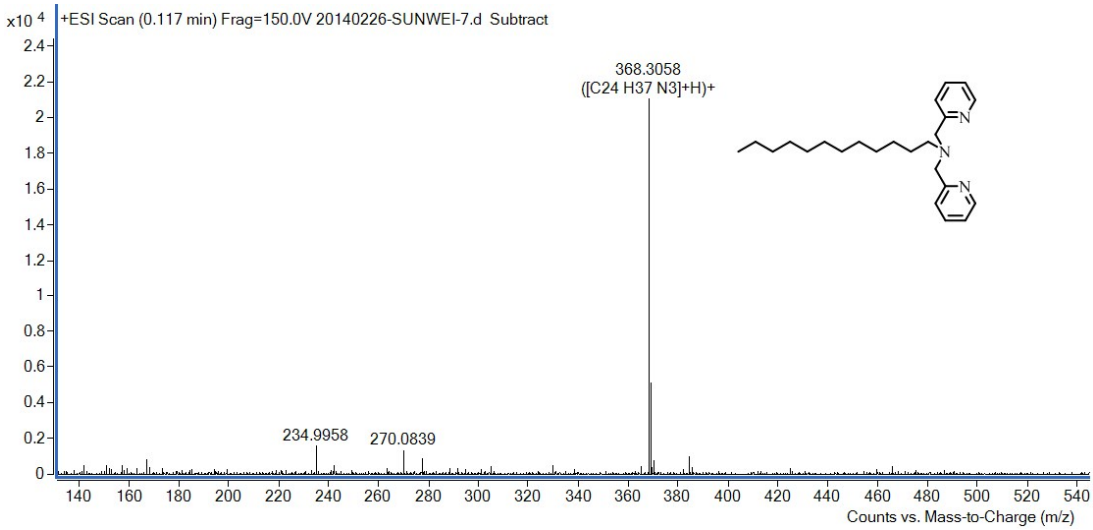


Fig. S14 HR-MS of compound **DPA-12**.

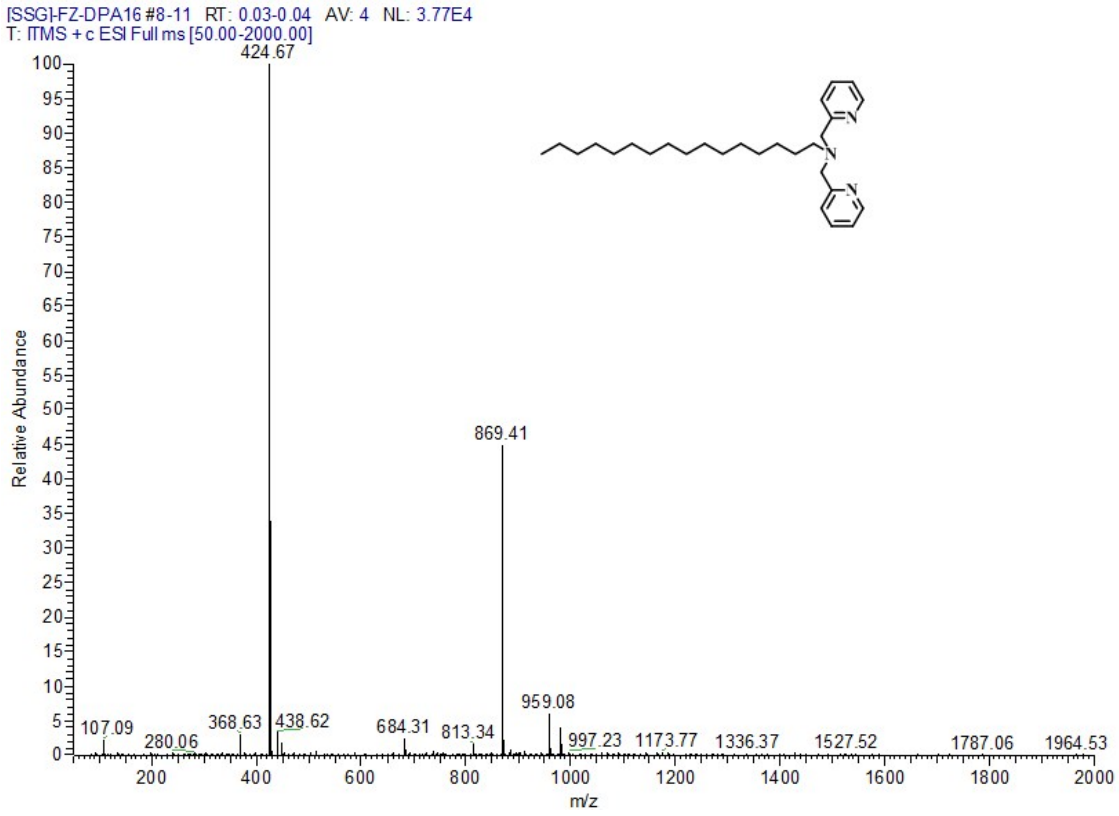


Fig. S15 MS of compound **DPA-16**.

S1 a) Y. Xu, A. Malkovskiy, Q. Wang and Y. Pang, *Org. Biomol. Chem.*, 2011, **9**, 2878; b) Y. Xu, Z. Li, A. Malkovskiy, S. Sun and Y. Pang, *J. Phys. Chem. B*, 2010, **114**, 8574.

S2 a) A. Neves, M. A. de Brito, V. Drago, K. Griesar and W. Haase, *Inorg. chim. acta*, 1995, **237**, 131; b) J. N. Hamann, M. Rolff and F. Tuczek, *Dalton trans.*, 2015, **44**, 3251.