

Supporting Information for “A Tetranaphthoimidazolium Receptor as a Fluorescence Chemosensor for Phytate†”

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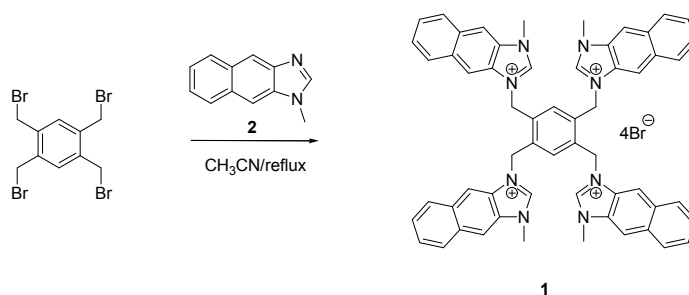
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Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Thin layer chromatography (TLC) was carried out using Merck 60 F₂₅₄ plates with thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F₂₅₄ plates with the thickness of 1 mm.

Melting points were measured using a Büchi 530 melting point apparatus. ¹H NMR and ¹³C NMR spectra were recorded using Bruker 250 MHz or Varian 500 MHz. Chemical shifts were given in ppm and coupling constants (*J*) in Hz. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).



Scheme S1. Synthesis of probe 1.

Synthesis of 2¹

NaH (330 mg, 8.3 mmol, 60% in mineral oil) was added to a mixture of 1H-naphtho[2,3-d]imidazole (690 mg, 4.1 mmol) in THF (20mL) at 0 °C. After the reaction mixture had been stirred for 20min at 0 °C, iodomethane (960mg, 6.3mmol) was added. After additional stirring for 1h at room temperature, water (50mL) was added to the reaction mixture and the mixture extracted with CHCl₃. The organic layer was separated, dried with anhydrous magnesium sulfate, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (CH₂Cl₂:MeOH=100:1) afforded 4 (680mg, 91%) as a pale-yellow solid; m.p. 152-154 °C. ¹H NMR (CDCl₃, 250 MHz) : δ 8.15 (s, 1H), 7.86 (m, 1H), 7.76 (m, 2H), 7.47 (s, 1H), 7.28 (m, 2H), 3.52 (s, 3H). ¹³C NMR (CDCl₃, 62.5 MHz) : δ 147.49, 143.80, 135.12, 130.49, 130.02, 128.55, 127.46, 124.46, 123.49, 117.20, 105.10, 30.96. HRMS (FAB) calcd for C₁₂H₁₁N₂ [M+H]⁺ 183.0844; found 183.0925

Synthesis of 1

A mixture of **2** (170 mg, 0.93 mmol) and 1,2,4,5-tetrakis(bromomethyl)benzene (100mg, 0.22mmol) in acetonitrile (10 mL) was heated at reflux for 24h under N₂. After cooling to room temperature, the precipitate was filtered and washed with cold CH₂Cl₂ to give **1** as a white solid (210 mg, 80 %). m.p. decompose >300 °C. ¹H NMR (DMSO-*d*₆-D₂O (9:1), 300 MHz) : δ 9.82 (s) 8.24 (s, 4H) 8.11 (s, 4H) 7.99-7.96 (d, 4H) 7.68-7.65 (d, 4H) 7.55-7.51 (m, 6H) 7.40-7.35 (t, 4H) 6.00 (s, 8H) 3.88 (s, 12H). ¹³C NMR (DMSO-*d*₆-D₂O (9:1), 62.5 MHz) : δ 146.84, 134.47, 133.45, 131.33, 131.17, 130.54, 129.84, 128.79, 128.37, 127.34, 127.20, 111.04, 110.97, 47.61, 33.84. HRMS (FAB) calcd for C₅₈H₅₀Br₃N₈ [M-Br]⁺ 1095.1703; found 1095.1709

Preparation of fluorometric anion solutions

Stock solutions (1 mM) of the IP₆ in distilled water were prepared. Stock solutions of **1** (0.1 mM) was also prepared in distilled water. Test solutions were prepared by placing 15 μL of the probe stock solution into a test tube, adding an appropriate aliquot of guest stock, and diluting the solution to 3 mL with 20 mM HEPES (pH 7.4). For all measurements, excitation was at 325 nm. Excitation and emission slit widths were 3nm/3nm.

Cell culture

HeLa cells (human epithelial adenocarcinoma) were obtained from Korean Cell Line Bank (Seoul, Korea). Cells were grown in RPMI 1640 (Roswell Park Memorial Institute) supplemented with 10 % fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. Cells were incubated at 37 °C in a 5 % CO₂ atmosphere. WA 38 VA-13 subclone 2RA cells (human lung epithelial normal cell) were obtained from Korean Cell Line Bank (Seoul, Korea). Cells were grown in EMEM (Eagle's minimum essential medium) in Hank's BSS with non-essential amino acids and pyruvic acid (1 mM) supplemented with 10 % fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. Cells were incubated at 37 °C in a 5% CO₂ atmosphere.

Confocal microscopy imaging

Cells were seeded in a 35-mm glass bottomed dishes at a density of 3 × 10⁵ cells per dish in culture media. After 24h, **1** (30 μM) (20 mM HEPES buffer, pH 7.4) added to the cells and the cells were incubated for 30 min at 37 °C. After washing with the DPBS twice to remove the residual probe, the cells were incubated with phytic acid for 30 min. After washing with the DPBS, cells were imaged by confocal laser scanning microscopy (LSM 510 META, Carl Zeiss, Germany). Cells were excited by a 405-nm diode laser, and fluorescence was detected at BP 420-480 nm.

Cytotoxicity test

HeLa cells were seeded in a 24-well plate with culture media. After 24 h, 0, 1, 5, 50 μM of **1** in the culture media were added to the cells and incubated for 24 h at 37 °C. After washing with the DPBS, cells were trypsinized with trypsin-EDTA solution and counted the number of live cells. Data are expressed as mean \pm standard deviation of three independent experiments.

Reference

1. Xu, Z.; Kim, S. K.; Han, S. J.; Lee, C.; Kociok-Kohn, G.; James, T. D.; Yoon, J. *Eur. J. Org. Chem.* **2009**, 3058.

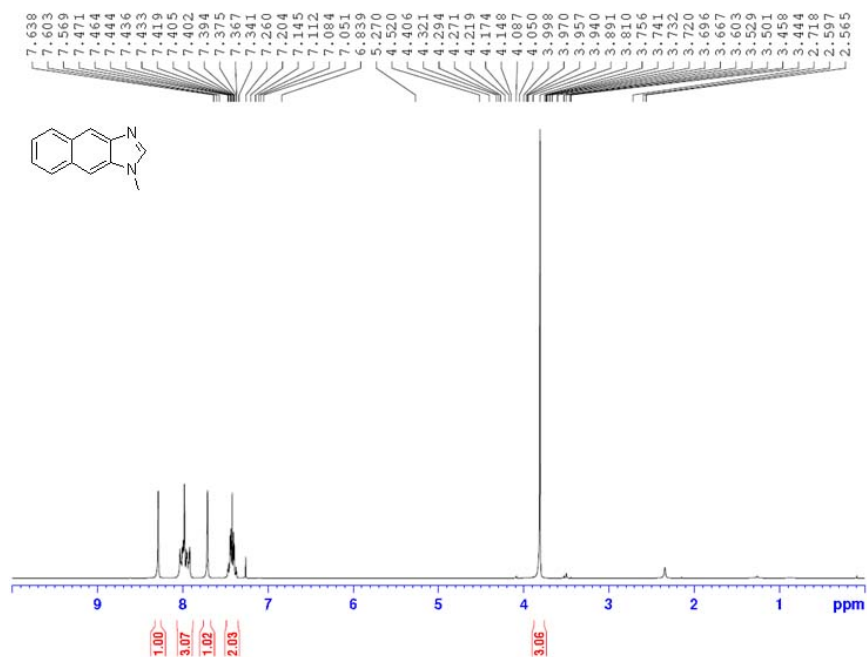


Fig. S1 ¹H NMR (250 MHz) of compound **2** in CDCl₃.

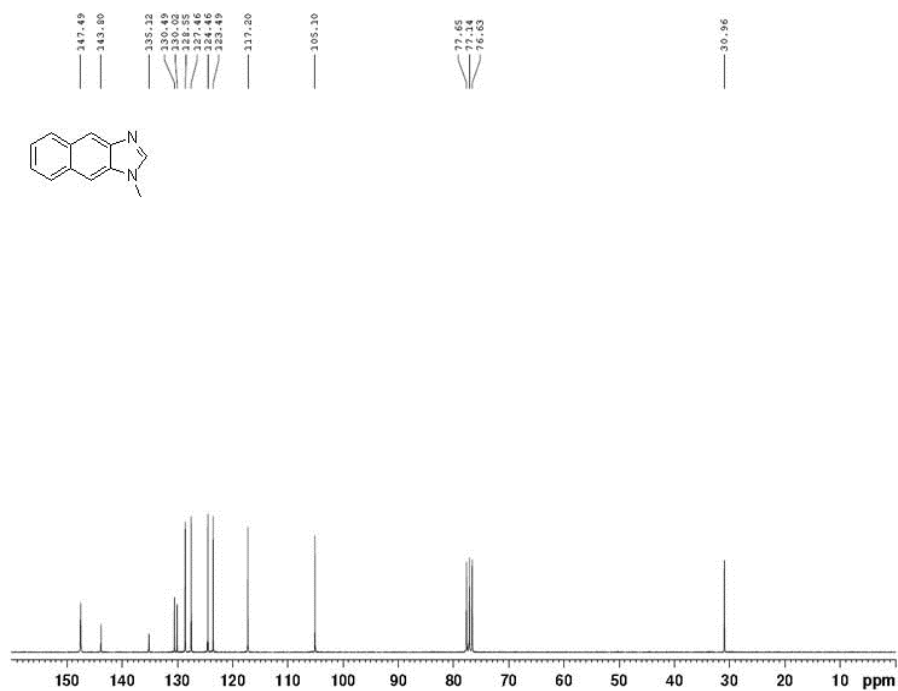


Fig. S2 ¹³C NMR (62.5 MHz) of compound **2** in CDCl₃.

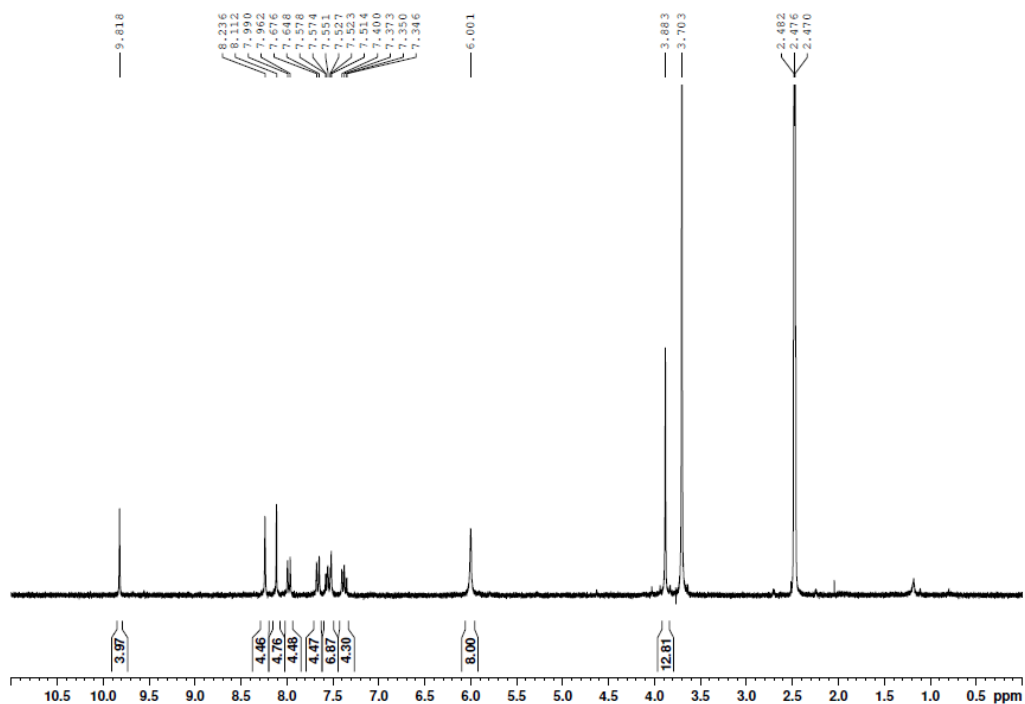


Fig. S3 ^1H NMR (300 MHz) of compound **1** in $\text{DMSO-}d_6\text{-D}_2\text{O}$ (9:1).

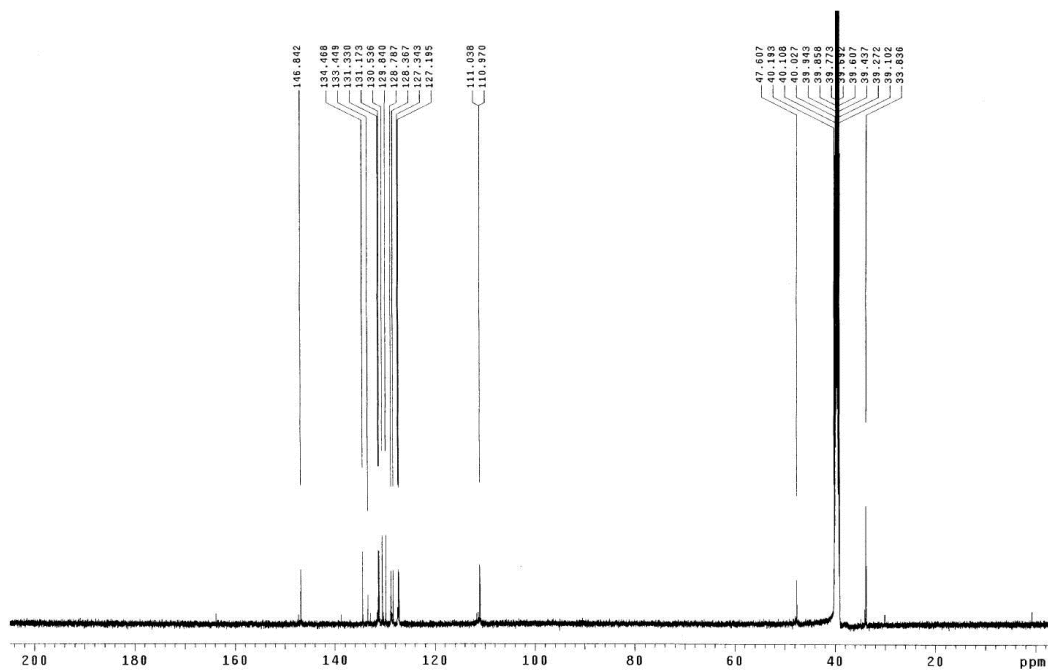


Fig. S4 ^{13}C NMR (62.5 MHz) of compound **1** in $\text{DMSO-}d_6\text{-D}_2\text{O}$ (9:1).

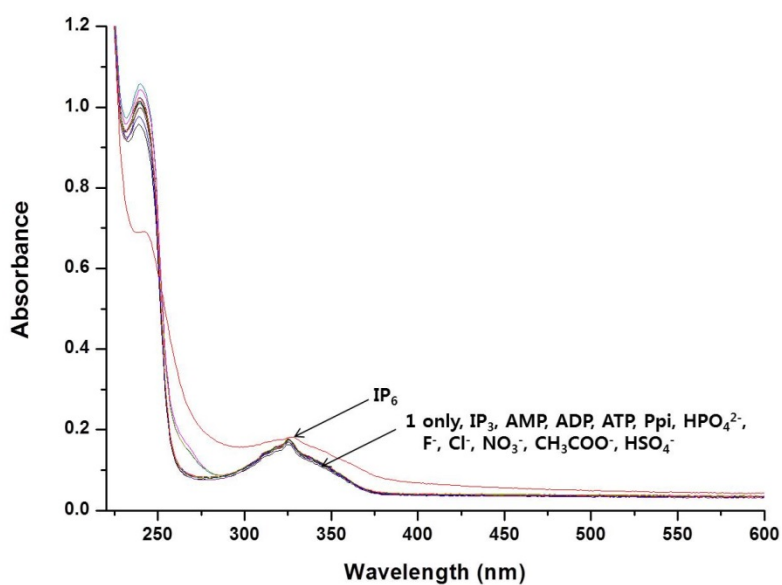


Fig. S5 UV spectra of **1** (5 μM) in HEPES buffer (0.02 M, pH 7.4) upon adding of 1 eq uiv. of anions.

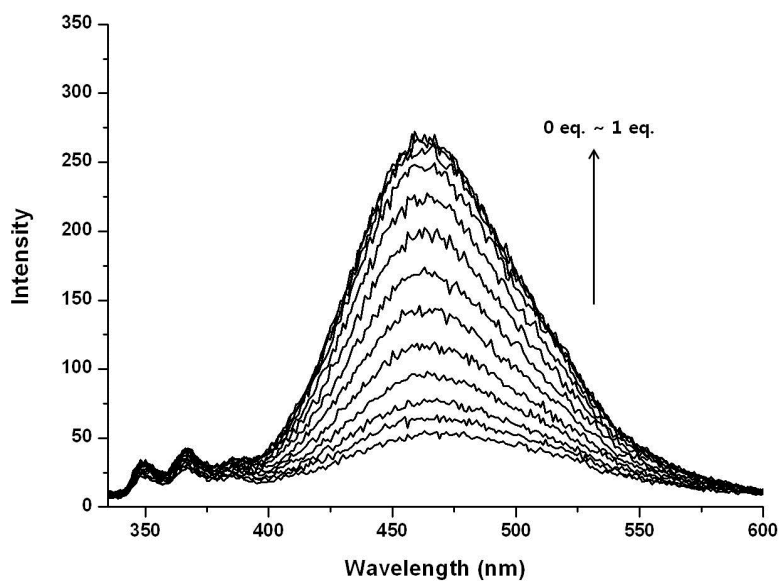


Fig. S6 Fluorescent titrations of **1** (5 μM) in HEPES buffer (0.02 M, pH 7.4) upon adding of 0-1 equiv. of IP_6 . ($\lambda_{\text{ex}} = 325 \text{ nm}$, Slit: 3 nm/3 nm)

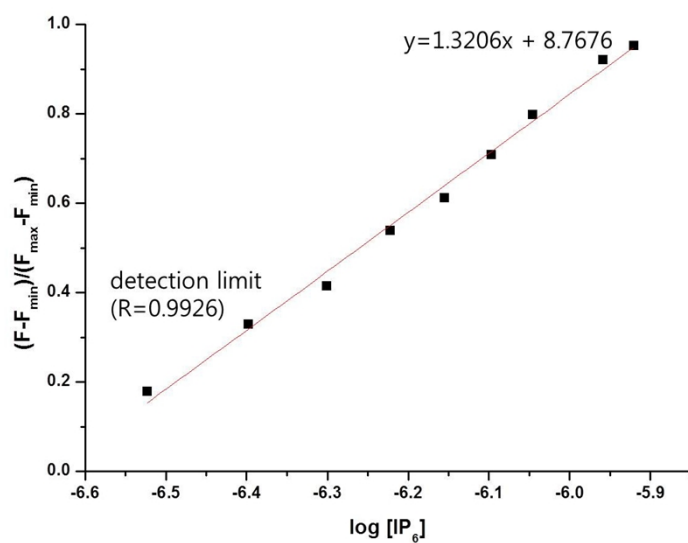


Fig. S7 Normalized fluorescence responses of **1** (1 μ M) to changing IP_6 concentrations in HEPES buffer (0.02 M, pH 7.4).

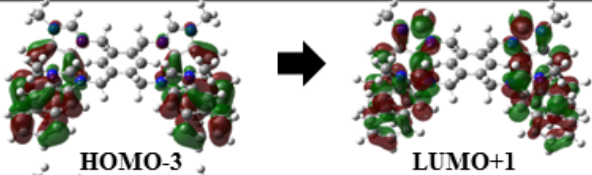
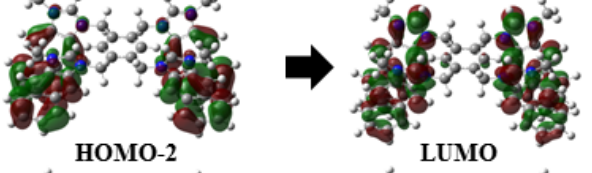
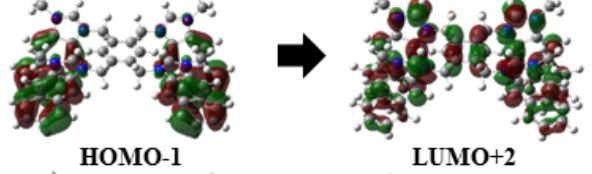
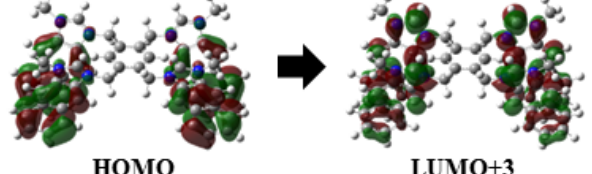
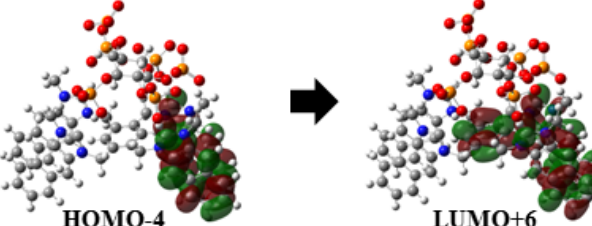
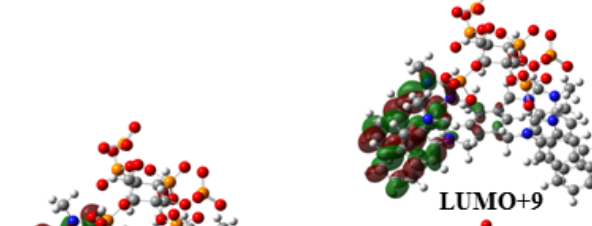
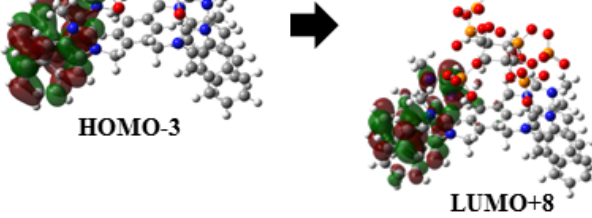
	Orbital Transitions	Percentage (%)
1	 HOMO-3 → LUMO+1	15 %
	 HOMO-2 → LUMO	16 %
	 HOMO-1 → LUMO+2	34 %
	 HOMO → LUMO+3	35 %
1+IP₆	 HOMO-4 → LUMO+6	12 %
	 HOMO-3 → LUMO+9	8 %
	 HOMO-3 → LUMO+8	80 %

Fig. S8 The important orbital transitions to the excitation and the corresponding orbital shapes for **1** and **1+IP₆**.

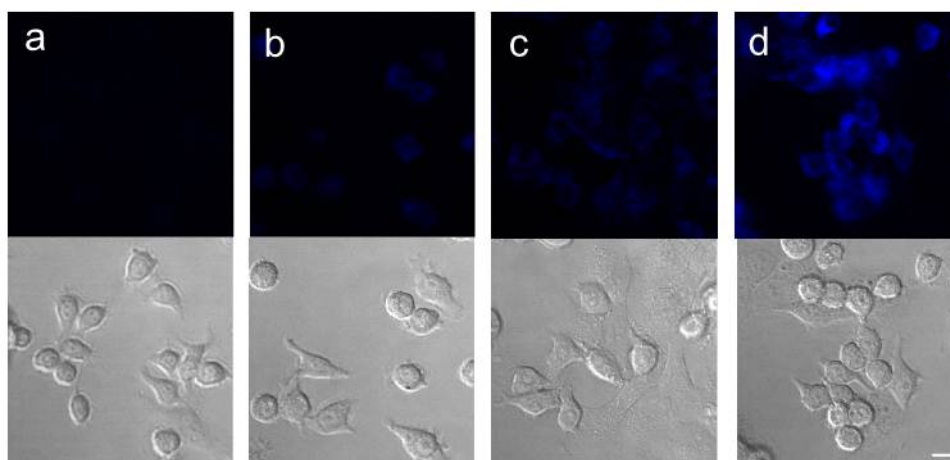


Fig. S9 Confocal fluorescence images of **1** in WI38 VA13 subclone 2RA cells. (a) no **1** (b) only **1** (30 μM) after 30 mins. (c) **1** (30 μM) and Phytic acid (IP_6) (5 μM) after 30 mins. (d) **1** (30 μM) and Phytic acid (IP_6) (50 μM) after 30 mins. Lower images: bright field d. ex 405/em BP 420-480 nm, scale bar: 10 μm .

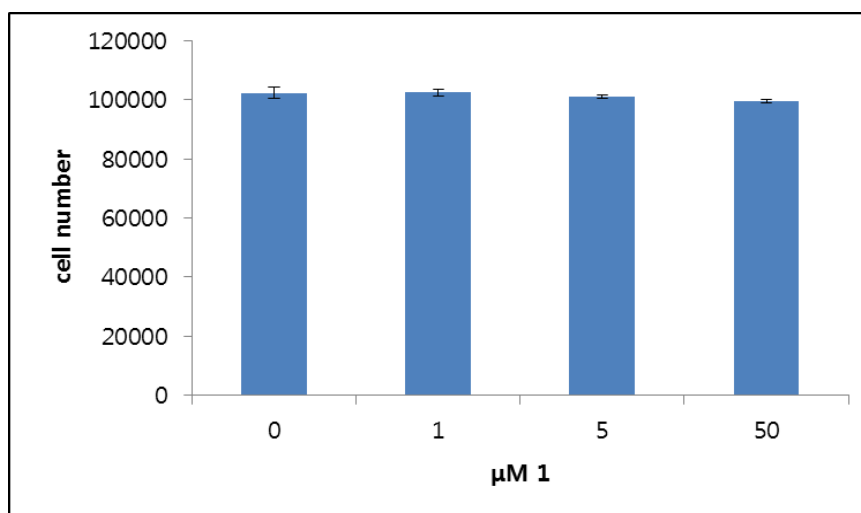


Fig. S10 Cell viability assay. HeLa cells were treated with 0, 1, 5, 50 μM of **1** for 24 h. After 24 h, the HeLa cells trypsinized with trypsin-EDTA and counted the live cell number. Results are expressed as mean \pm standard deviation of three independent experiments.