Supporting Information

A water-soluble two-photon fluorescence chemosensor for ratiometric imaging mitochondrial viscosity in living cells

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Contents

Materials and Measurements:

Crystallographic Data Collection and Refinement: The single-crystal X-ray data were collected with a Bruker SMART APEX II CCD instrument with graphite monochromatic Mo-K_α radiation (λ = 0.71073 Å) at 298K. The data restorations and sets were corrected by the SAINT and SADABS programs respectively. The structure of sensor **EIN** was solved by direct methods and refined by full-matrix least-squares methods with the SHELX-97 program package. All the non-hydrogen atoms were refined anisotropically while hydrogen atoms attached to the carbon and oxygen atoms were placed in the calculated positions and in the riding model approximation. The crystallographic details are provided in **Table S1** and **Fig. S1**. The selected bond lengths and angles are given in Supporting Information, **Table S2**. Crystallographic data have been deposited at the Cambridge Crystallographic Data Center, the numbers is 1449562.

Cytotoxicity test: HepG2 cells growing in the log phase were seeded into 96-well plates ($\sim 1\times10^4$ cells/well) and allowed to adhere for 24h. **EIN** stock solutions were diluted by fresh mediumin to desired concentration (10, 20, 30, 40, 50 μM). The cell medium was then exchanged by different concentrations of **EIN** medium solutions. They were then incubated at 37 °C in 5% CO_2 for 24 h before cell viability was measured by the MTT assay. The cell medium solutions were exchanged by 100 μL of fresh medium, followed by the addition of 10 μL (5 mg / mL) MTT solution to each well. The cell plates were then incubated at 37 °C in 5% CO_2 for 4 h. Absorbance was measured at 450 nm. The absorbance measured for an untreated cell population under the same experimental conditions was used as the reference point to establish 100% cell viability. Duplicated experiments have been tested.

Cell culture and staining: For HepG2 cells (liver hepatocellular carcinoma, ATCC No. HB-8065), the medium used was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS, GIBCO), penicillin and streptomycin, L-glutamine and fungizone. For live cell confocal laser scanning microscopy experiment, HepG2 cells were seeded in 24-well glass bottom plate (In Vitro

Scientific, P24-1.5H-N) at density of 10,000, and incubated for 72-96 hours at 37 °C in 95% air 5% CO_2 in order to allow the cells to reach ~90% confluence, the medium changed every two days. **EIN** was prepared as 1mM glycerol solution, and dilute in culture medium at 10 μM for 30 min at 37 °C in 95% air 5% CO_2 and then imaged with confocal microscopy. For colocalization experiments, **EIN** (10 μM) was incubated with HepG2 cells in DMEM for 30 min, and then the medium were replaced with fresh medium in the presence of Mito-Tracker Red (1 μM) or Lyso-Tracker Blue (1 μM) for 30 min. The 24-well plate were washed by PBS twice and imaged.

Table S1. Crystal data and structure refinements for **EIN**.

Complex	**EIN**
Chemical Formula	$C_{27}H_{33}F_6N_2O_4P$
Formula Mass	594.52
Crystal system	triclinic
Space group	$P\bar{1}$
a/Å	10.820(6)
b/Å	11.364(7)
c/Å	12.581(7)
β /º	91.648(7)
V(Å^3)	1424.9(14)
Temperature/K	296
Absorption coefficient, μ/mm^{-1}	0.171
No. of reflections measured	5049
No. of independent reflections	2318
R_{int}	0.0469
No. of formula units per unit cell ,*Z*	2
R_1, wR_2 [I ≥2σ (I)]	0.0829, 0.2277
R_1, wR_2 [all data]	0.1751, 0.2624

GOF	1.105
Largest diff. peak and hole($eÅ^{-3}$)	0.777, -0.402

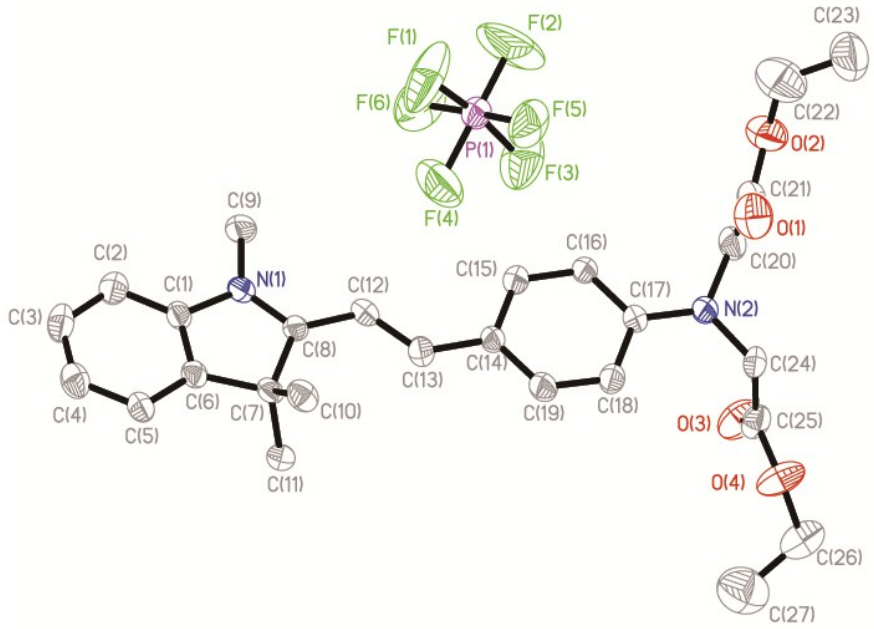


Fig. S1 The asymmetric unit of **EIN** with displacement ellipsoids at the 50% probability level. H atoms have been omitted for clarity.

Table S2. Selected bond lengths /Å and angles /° for **EIN**.

C(8)-N(1)	1.318(5)	C(17)-N(2)	1.376(6)
C(21)-O(1)	1.183(7)	C(25)-O(3)	1.188(7)
C(1)-C(6)	1.372(5)	C(12)-C(13)	1.345(6)
F(5)-P(1)	1.551(5)	C(9)-N(1)	1.451(5)
C(9)-N(1)-C(8)	127.76(3)	O(3)-C(25)-C(24)	126.05(5)
C(8)-C(12)-C(13)	123.78(4)	O(4)-C(25)-O(3)	122.07(5)
C(24)-N(2)-C(20)	117.31(4)	C(23)-C(22)-O(2)	117.50(1)
C(1)-C(1)-C(6)	123.86(4)	F(2)-P(1)-F(5)	90.22(3)

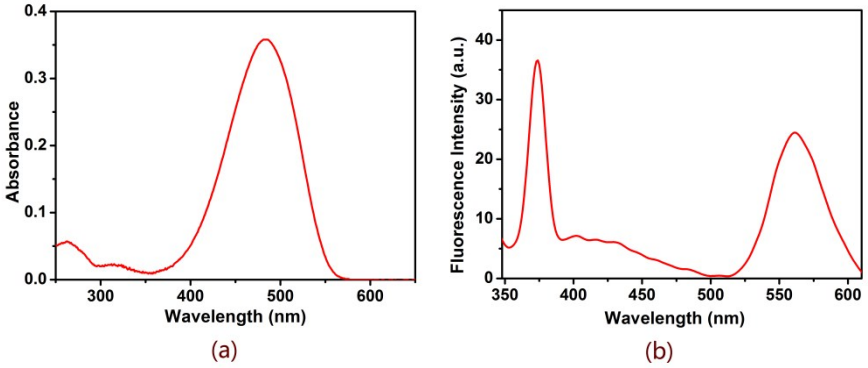


Fig. S2 The linear absorption (a) and emission spectra (b) of **EIN** in H_2O buffered with HEPES.

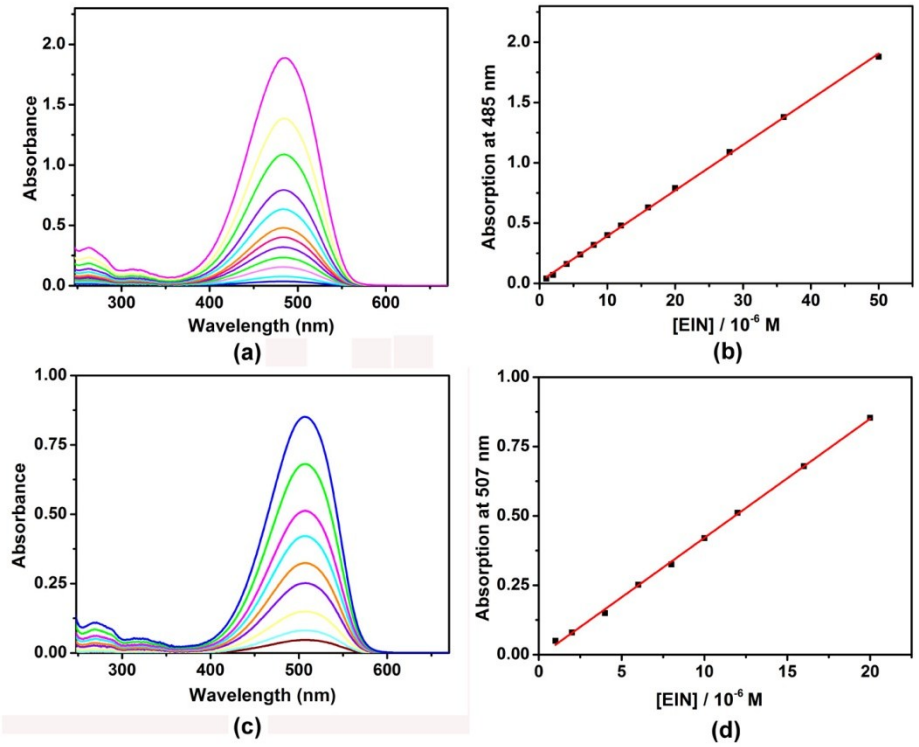


Fig. S3 (a) and (b) Absorption spectra and plot of intensity against the concentration of **EIN** in H_2O buffered with HEPES. (c) and (d) Absorption spectra and plot of intensity against the concentration of **EIN** in pure glycerol solution.

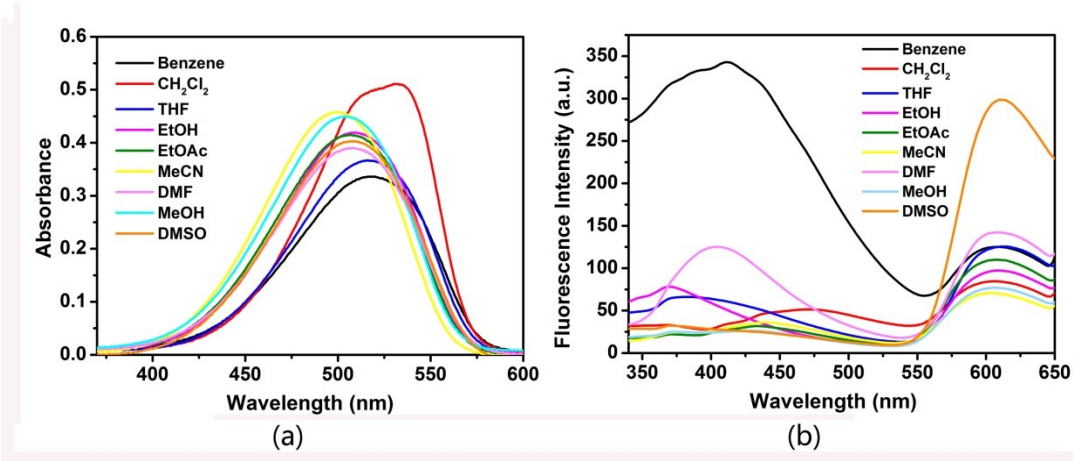


Fig. S4 The linear absorption (a) and emission spectra upon excited at 330 nm (b) of **EIN** in different solvents with different polarities with a concentration of 10 μM.

Table. S3 Single-photon-related photophysical properties of **EIN** in different solvents.

Solvents	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Stokes Shift(nm)
Benzene	516	412, 607	82, 277
CH_2Cl_2	531	470, 608	140, 278
THF	516	387, 613	48, 283
EtOH	510	369, 609	39, 279
EtOAc	507	433, 607	103, 277
MeCN	499	440, 603	110, 273
DMF	506	404, 607	74, 277
MeOH	504	428, 605	98, 275
DMSO	507	371, 610	41, 280

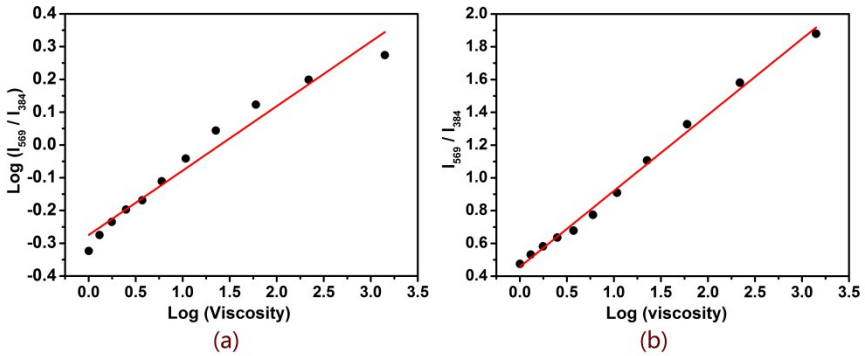


Fig. S5 (a) The linear response between log (I_{569} / I_{384}) and log (viscosity) in the water / glycerol solvent (R^2 = 0.960); (b) The linear response between I_{569} / I_{384} and log (viscosity) in the water / glycerol solvent (R^2 = 0.994).

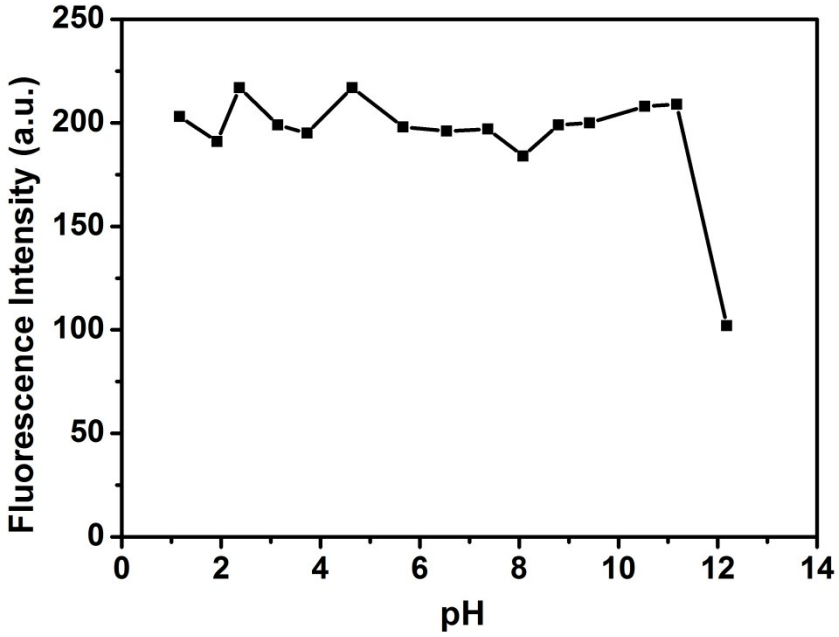


Fig. S6 The fluorescent intensities for **EIN** at varied PH values.

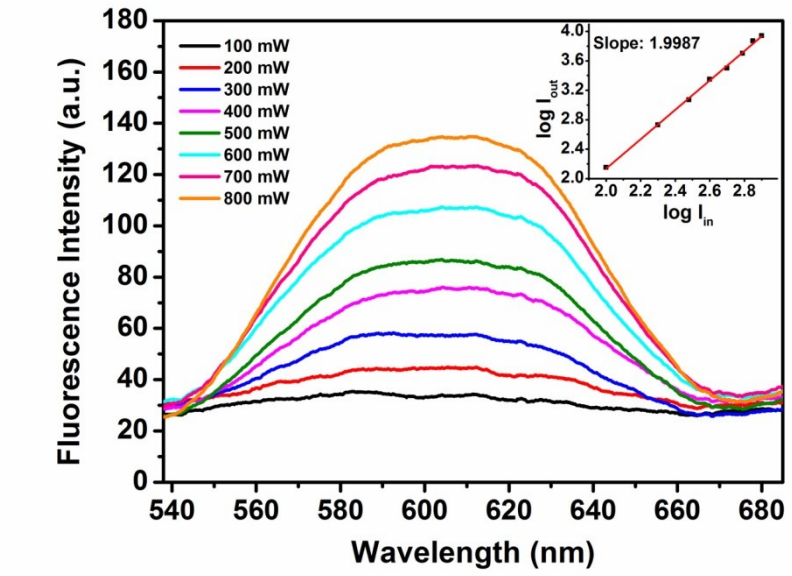


Fig. S7 The two-photon fluorescence spectra of **EIN** excited by the different input laser power at the optimum wavelength, the inset shows the two-photon absorption verification which I_{in} and I_{out} represent the input laser power and output fluorescence, respectively.

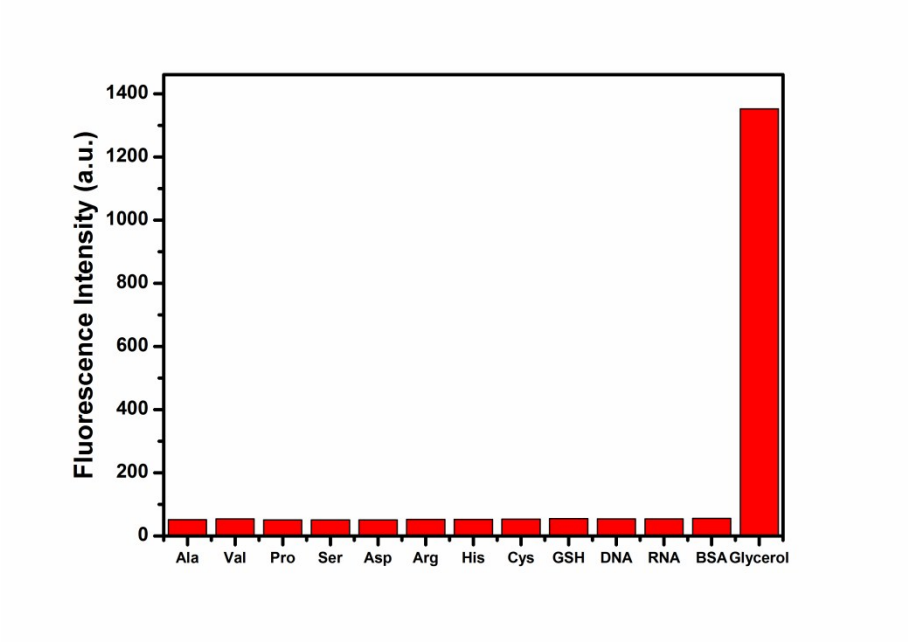


Fig. S8 The fluorescence spectra titration of **EIN** (10 μM) in pure HEPES buffer (pH = 7.4) by Ala (100 μM); Val (100 μM); Pro (100 μM); Ser (100 μM); Asp (100 μM); Arg (100 μM); His (100 μM); Cys (100 μM); GSH (100 μM); DNA (200 μM); RNA (200 μM); BSA (200 μM) and 99.9 % glycerol during 120 min (excited at 490 nm).

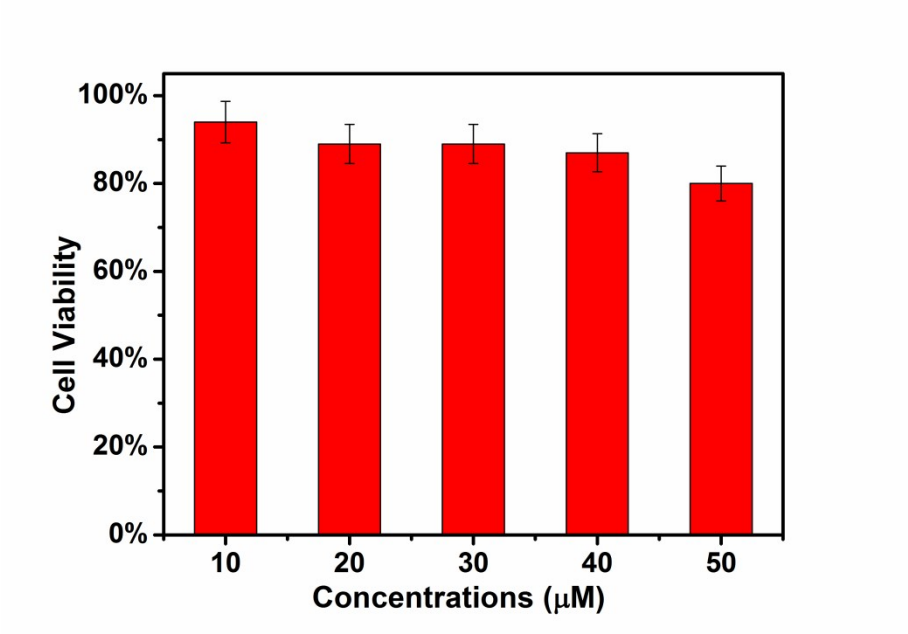


Fig. S9 Cytotoxicity data results of **EIN** obtained from the MTT assay of HepG2 cells.

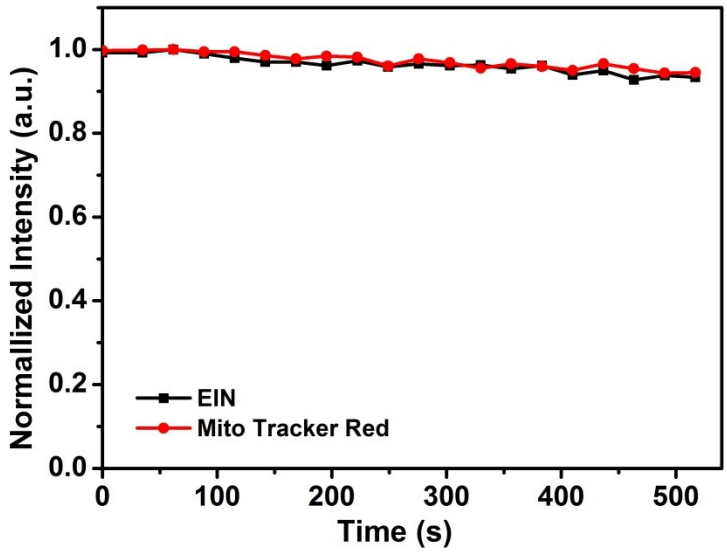


Fig. S10 Photostability experiments of **EIN** and Mito Tracker Red with 10 μM, respectively.

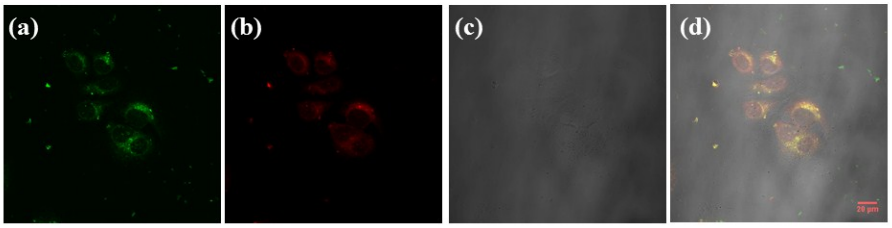
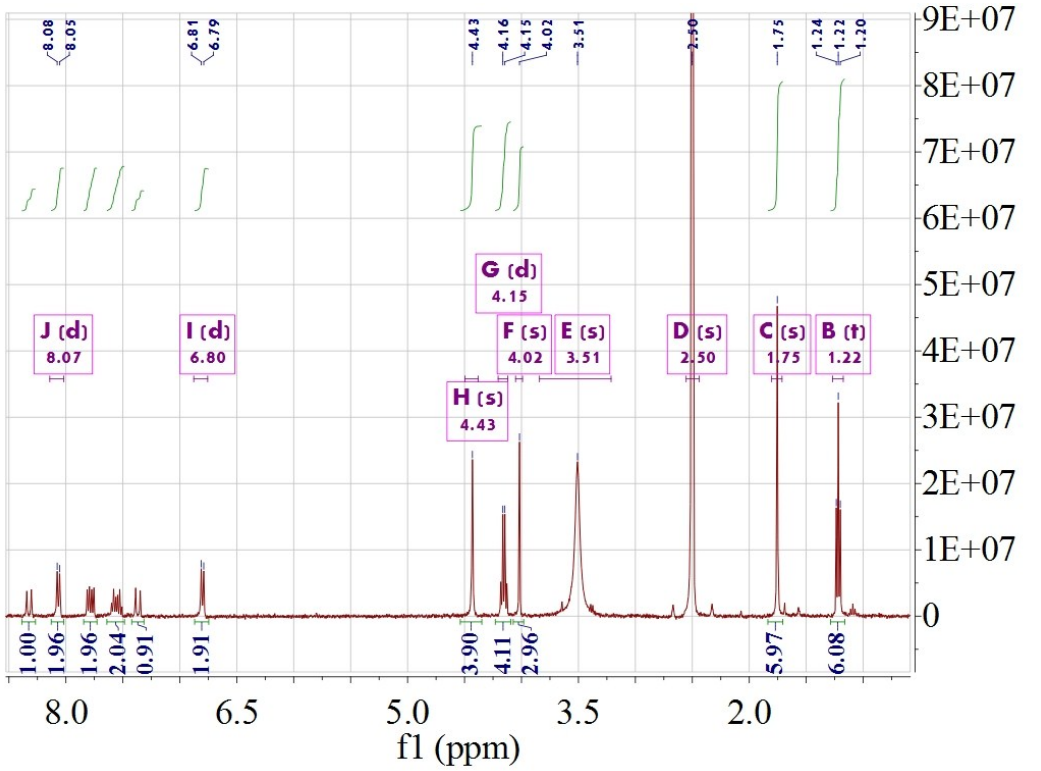


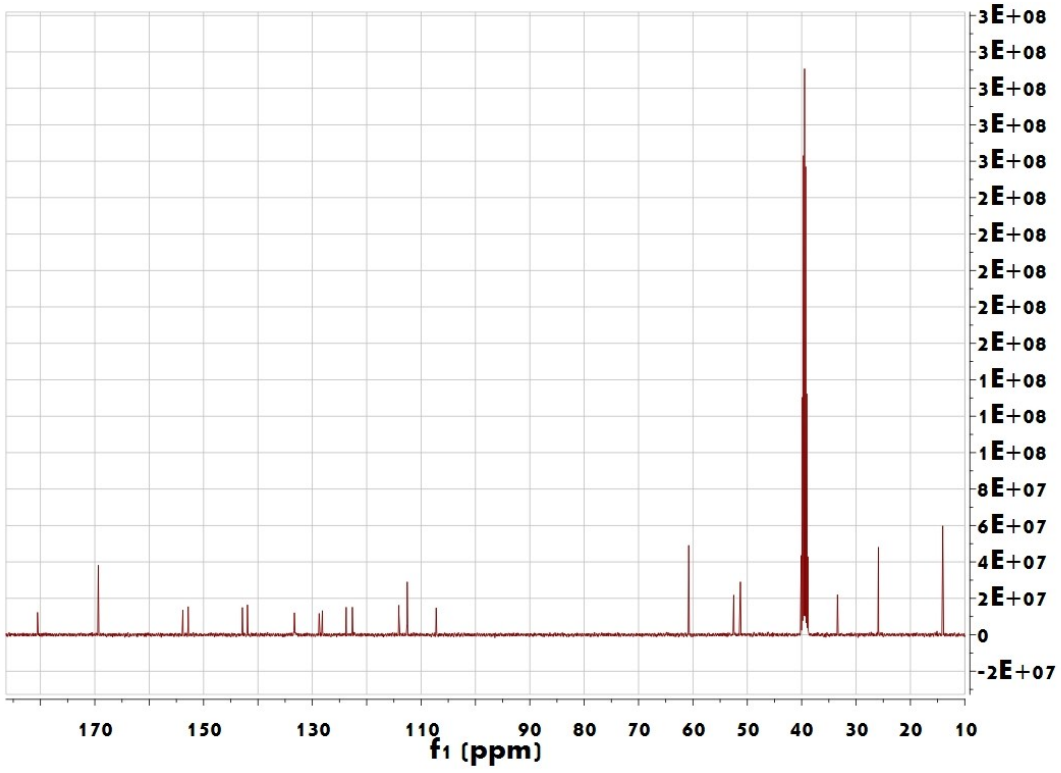
Fig. S11 Confocal laser fluorescence microscopic images of HepG2 cells treated with 10 μM **EIN**; (a) Green channel fluorescence image detected at 520-580 nm upon excited at 488 nm; (b) Green channels fluorescence images detected at 520-580 nm upon excited at 840 nm; (c) Bright fields of HepG2 cells; (d) Merged image of (a), (b) and (c).

1. R. C. Weast, *CRC Handbook of Chemistry and Physics*, CRC Press Inc., Boca Raton, FL, USA, 68th ed., 1987, D221-D269.

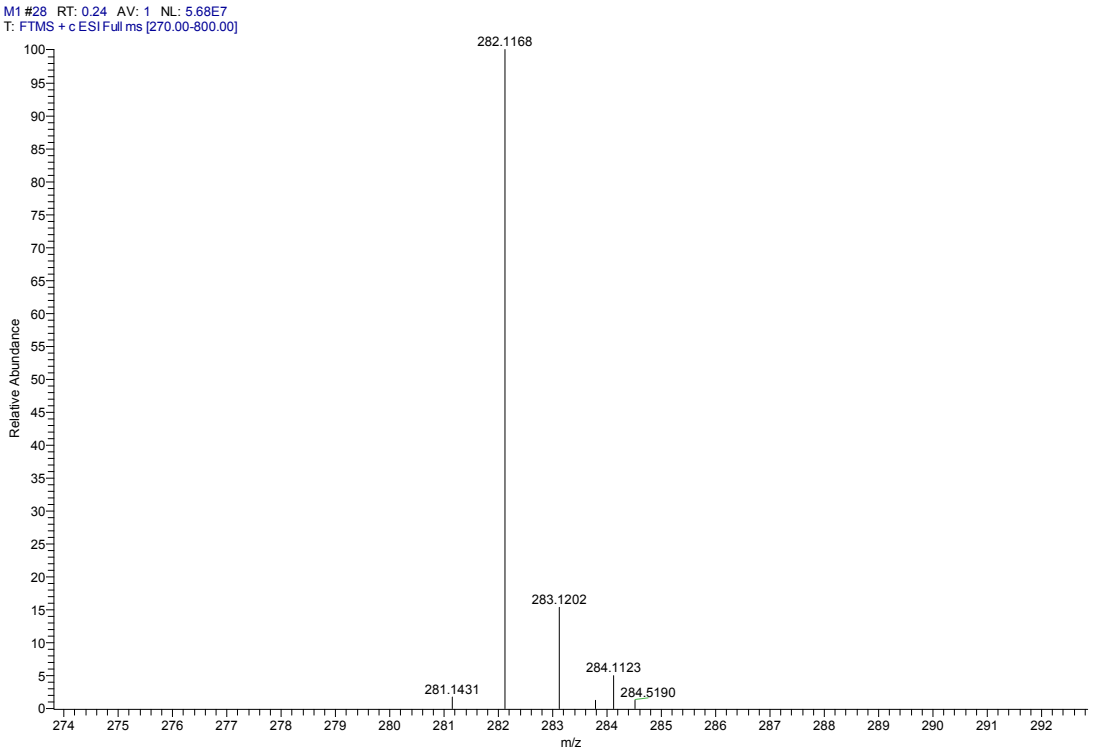
^{1}H-NMR spectra of **EIN**



^{13}C-NMR spectra of **EIN**



HR-MS of **EIN**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283.1202
284.1123
281.1431
284.5190
Relative Abundance
m/z