Supporting Information

A Golgi-targeted viscosity rotor for monitoring early Alcohol-Induced Liver Injury

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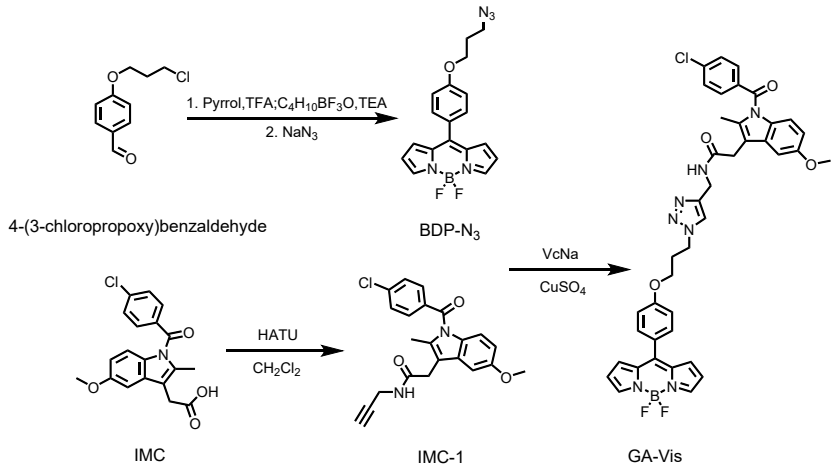
1. Experimental procedures

1.1 Materials and apparatus

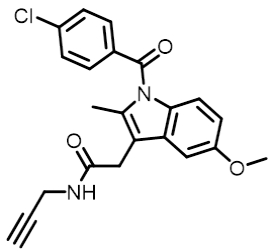
Unless otherwise noted, all regents were obtained from commercial suppliers and used without further purification. Column chromatography was performed with silica gel (200-300 mesh). ^{1}HNMR and ^{13}CNMR spectra were collected in $CDCl_3$, DMSO-d_6 with TMS as internal reference (Cambridge Isotope Laboratories) on Bruker 400 MHz spectrometer or AVANCE III 500 MHz spectrometer. HRMS was performed on a Waters Synapt G2-Si HRMS mass spectrometer. Fluorescence spectra were measured on the Agilent spectrometer. Fluorescence lifetime were performed using the TCSPC technique on HORIBS Jobin Yvon IBN photo counting fluorescence system with nano LED laser excitation at 455 nm. Fluorescent images were obtained on the confocal microscopy (Olympus FV1000). Fluorescence lifetime imaging was performed on a DCS 120 Multiphoto FLIM System.

1.2 Synthetic procedures



Scheme S1. The synthesis process of **GA-Vis**.

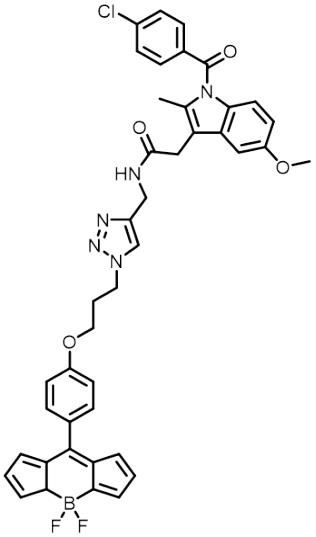
BDP-N_3 was synthesized according to methods.[1]



Scheme S2. The chemistry structure of IMC-1.

Synthesis of IMC-1.[2] Indomethacin (2 g, 5.59 mmol) and HATU (2.55 g, 6.71 mmol) were dissolved in dichloromethane (20 mL), and triethylamine (1.6 mL, 11.18 mmol) was added under

stirri for 2 h. Then propylamine (2 g, 7.24 mmol) was added, and the reaction was continued to stir at room temperature for 5 h. After completion of the reaction, 100 mL of water was added, extracted 3 times with dichloromethane, and the solvents were removed under reduced pressure and the product was repeatedly chromatographed (PE / EA = 1: 3) to IMC-1 as a pale yellow solid in 71% yield. ^{1}H NMR (400 MHz, DMSO) δ 8.47 (t, *J* = 5.3 Hz, 1H), 7.67 (dd, *J* = 19.9, 8.5 Hz, 4H), 7.12 (d, *J* = 2.3 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.71 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.87 (dd, *J* = 5.3, 2.3 Hz, 2H), 3.77 (s, 3H), 3.54 (s, 2H), 3.09 (t, *J* = 2.3 Hz, 1H), 2.24 (s, 3H).



Scheme S3. The chemistry structure of **GA-Vis**.

Synthesis of **GA-Vis**. IMC-Alk (100 mg, 274.59 μmol), BDP-N_3 (119 mg, 302.05 μmol) were dissolved in the mixed solvent of THF/EtOH (1:1, 6 ml), then CuSO4•5H2O (69 mg, 274.59 μmol) and sodium ascorbate (109 mg, 108.79 μmol) were dissolved in H_2O (0.1 ml) and added to the solution. The reaction mixture was stirred for 3.5 h at room temperature. Then the solvent was evaporated off under reduced pressure to give the crude product, which was purified by silica gel flash chromatography (EA) to afford **GA-Vis** as an orange solid（0.17 g, yields 83%). 1H NMR (400 MHz, DMSO) δ 8.55 (t, J = 5.5 Hz, 1H), 8.10 (s, 2H), 7.93 (s, 1H), 7.65 (dd, J = 16.5, 8.3 Hz, 6H), 7.14 (d, J = 8.5 Hz, 2H), 7.10 (d, J = 2.3 Hz, 1H), 7.06 (d, J = 4.1 Hz, 2H), 6.94 (d, J = 8.9 Hz, 1H), 6.73 – 6.67 (m, 3H), 4.54 (t, J = 6.8 Hz, 2H), 4.33 (d, J = 5.6 Hz, 2H), 4.10 (t, J = 5.8 Hz, 2H), 3.73 (s, 3H), 3.54 (s, 2H), 2.35 – 2.25 (m, 2H), 2.22 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.22, 168.28, 160.83, 156.21, 147.16, 143.56, 139.49, 136.30, 134.82, 133.63, 132.47, 131.30, 131.00, 130.31, 129.23, 126.78, 122.59, 118.36, 115.16, 114.50, 112.66, 112.01, 101.12, 64.36, 55.77, 47.04, 35.04, 32.16, 29.80, 13.39. HRMS (ESI) calcd for $C_{42}H_{36}BClF_2N5O_4$ 761.2495, found 761.2532.

1.3 Photo physical properties

GA-Vis was dissolved in DMSO to prepare the stock solution (1.0 mM). The probe was added to a 3 mL solution (different ratio of methol and glycerol, v/v), which were sonicated for 10 min to eliminate air bubbles. The final concentration of **GA-Vis** was 2 μM. Fluorescence spectrum measurements were carried out in this series of solutions. The viscosity values were recorded and measured by the viscometer. Change the ratio of solutions and increase the ratio of glycerol will cause an increase in viscosity from 1.8 cP (10% glycerol) to 950 cP (99% glycerol). The fluorescence emission was recorded ranging from 470 to 650 nm, with excitation of 460 nm. According to the Forster−Hoffmann equation, the quantitative relationship between I_{512} of **GA-Vis** and the solvent viscosity can be described as follows:

$$\log I = c + x \log \eta \quad (1)$$

$$\log \tau = c + x \log \eta \quad (2)$$

where I (eq 1) is the emission fluorescence intensity, η is the solvent viscosity, c is a constant, and x represents dye-dependent constant; τ (eq 2) is the fluorescence lifetime, c is a constant, and x is the sensitivity of the fluorescent probe to viscosity. A series of interference species were added to solutions, Ca^{2+}, Cu^{2+}, K^{+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Cysteine, Alanine, Lysine, Glucose, Glutathione, Human Serum Albumin, H_2O_2, and ·OH in ultrapure water to demonstrate the selectivity of probe.

1.4 Culture of cells and fluorescence imaging

LO2, HepG2 and HEK-293 cells were obtained from the institute of Basic Science (IBMS) of the Chinese Academy of Medical Sciences (CAMS). The LO2, HepG2 and HEK-293 cells were cultured in 1640, DMEM, MEM which supplemented with 10% fetal calf serum and streptomycin (0.1 mg/mL) at 37 °C in 95% air with 5% CO2, respectively. All cells in the exponential phase of growth were grown on 35 mm glass-bottom culture dishes (Φ 20 mm) for 24 - 36 h to reach 80% confluency. The cells were incubated with **GA-Vis** (2 μM) for 30 min at 37 °C, then cells were washed three times with PBS (pH=7.4). The image was recorded with 60× objectives using confocal microscopy (Olympus FV1000). The excitation wavelength was set to 488 nm for **GA-Vis**, with an emission-signal 500 – 550 nm. Figures were processed with FV10-ASW Viewer (Olympus) and Image J. Then the same image of the field of vision was recorded using fluorescence lifetime imaging microscopy (DCS 120 Multiphoto FLIM System). Figures were processed with Spcm64 and Image J.

1.5 Biochemical analyse of triglyceride

LO2 cells were collected for the detection of the level of triglyceride (TG) by using commercial assay kits according to the protocols from the manufacturer (Wuhan Elabscience Biotechnology Co., Ltd, Wuhan, China).

1.6 Golgi localization expiration

Grow LO2, HepG2 and HEK-293 cells in the exponential phase of growth on 35-mm glass-bottom culture dishes for 24 - 36 h to reach 70 - 90% confluency. These cells were used in co-localization experimentation. The cells were incubated with 1 mL 1640, DMEM, MEM containing **GA-Vis** (2 μM) for 30 min at 37 °C. Wash cells thrice with PBS and then add 1 mL 1640, DMEM, MEM culture medium containing 1 μM Golgi Tracker Red for 30 min. HEK-293 cells were incubated with 1 mL MEM containing **GA-Vis** (2 μM) for 30 min at 37 °C. Wash cells thrice with PBS and then add 1 mL 1 MEM culture medium containing 1 μM ER Tracker Red for 30 min. The cells were washed three times with PBS and observed under confocal microscopy (Olympus FV1000).

1.7 MTT assays

LO2 or HepG2 cells seeded at a density at 10000 cells per well into 90-well micro plates, and incubated for 24 h. After changing the fresh medium, incubate with different concentrations **GA-Vis** (1, 2, 4, 8, 16 μM) at 37 °C for 24 h. Subsequently, the culture medium was removed, 150 μL DMSO was added to dissolve the formazan crystals. The absorbance of solution was measured at 490 nm with 5 min gentle agitation using a TRITURUS microplate reader.

1.8 Culture of zebrafish

The AB line zebrafish embryo was obtained from Shanghai FishBio Co. Ltd. and incubated with embryo culture fluid under 28.5 °C. Zebrafish embryo culture medium was changed daily, and following the 1.4:1 contrast ratio of light to dark was cultured. All animal experiments were basically in line with the “National Laboratory Animal Management Regulations”.

The zebrafish embryo were transferred to six-well micro plates and incubated with E3 embryo media (pH 7.5; 0.7 mM $NaHCO_3$, 0.15 mM KH_2PO_4, 15 mM NaCl, 0.05 mM Na_2PO_4, 1.3 mM $CaCl_2$, 0.5 mM KCl, and 5% methylene blue). Zebrafish embryo were cultured to 36 hpf and transferred to six-well micro plates with 10 tails per well.

The embryos were incubated with E3 embryo media with 1% EtOH and replaced every 12 h to maintain same EtOH concentration. After 72 h, zebrafish were culture with E3 embryo medium containing **GA-Vis** (5 μM) for 1hours at 28.5 °C. Fish were washed three times with E3 embryo medium and anesthetized with MS-222. The image was recorded with 4× objective using confocal microscopy (Olympus FV1000). The excitation wavelength was set to 488 nm for **GA-Vis**, with an emission-signal range of 500-550 nm. And the fluorescence lifetime image was with 4× objective using fluorescence lifetime image (DCS 120 Multiphoto FLIM System). The excitation wavelength was set to 488 nm for **GA-Vis**, with an emission-signal range of 500-550 nm. Figure were processed with FV10-ASW Viewer (Olympus), Spcm64 and Image J.

2. Supplementary figures and tables

Table S1 The viscosity values corresponds to the glycerol percent volume (25 ℃)

the glycerol percent volume	the viscosity value (cP)
10%	1.8
20%	4.8
30%	7.7
40%	13
50%	28
60%	58
70%	130
80%	250
90%	630
99%	950

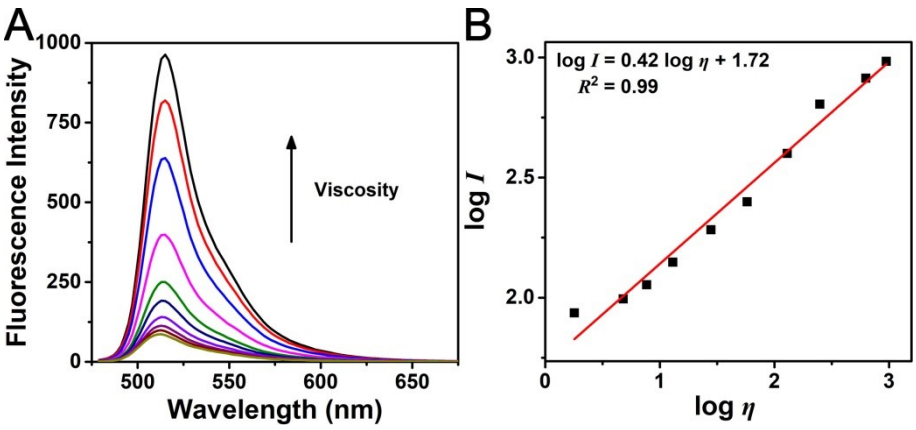


Fig. S1 (A) Fluorescence emission spectra of **GA-Vis** (2 μM) under different viscosities in the methanol/glycerol solutions. (B) Linear relationship between fluorescence intensity and viscosity.

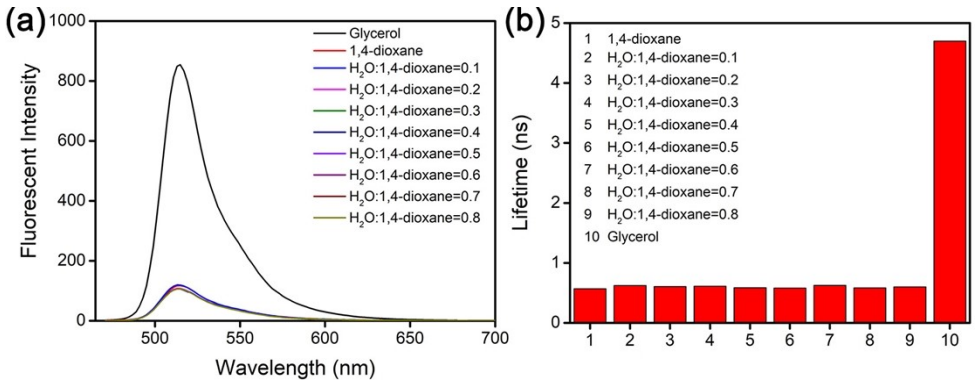


Fig. S2 (a) Fluorescence emission spectra of **GA-Vis** (2 μM) in different solvents, excited at 460 nm; (b) the fluorescence lifetime of **GA-Vis** (2 μM) responses to different polar solution, excited at 488 nm.

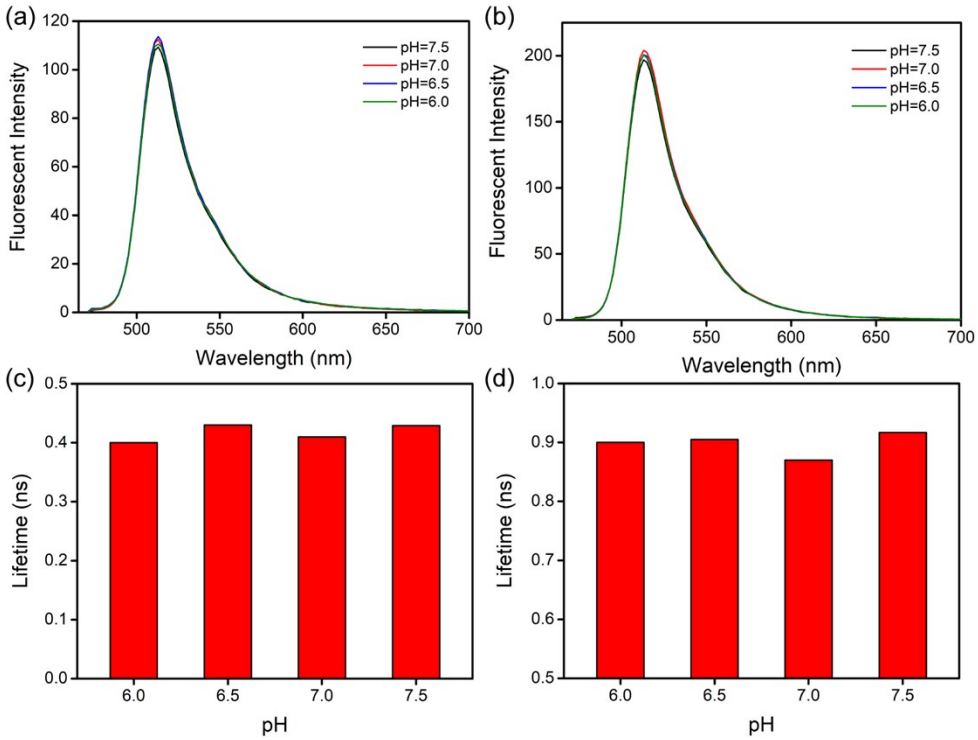


Fig. S3 (a, b) Fluorescence spectra of **GA-Vis** (2 μM) at different pH in methanol/glycerol mixtures at two viscosities: (a) 7.7 cP and (b) 58 cP; (c, d) Fluorescence Lifetime of **GA-Vis** (2 μM) at different pH in methanol/glycerol mixtures at two viscosities: (c) 7.7 cP and (d) 58 cP, excited at 488 nm.

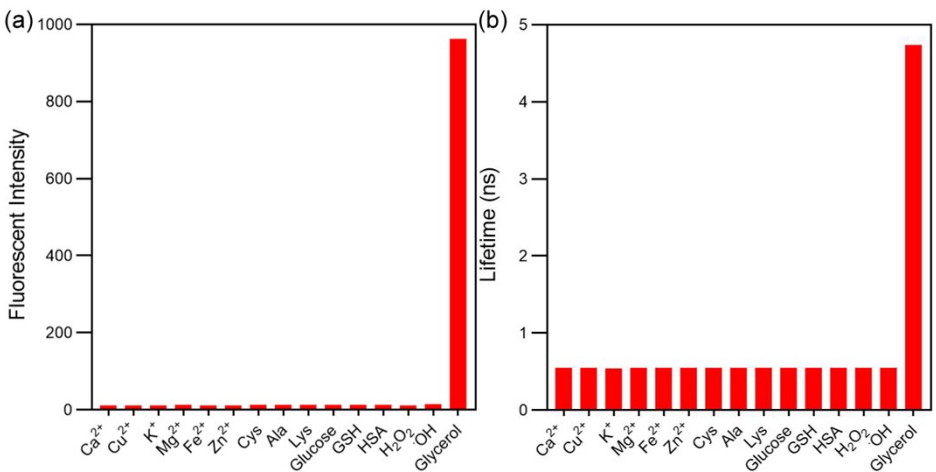


Fig. S4 (a) Fluorescent intensity of **GA-Vis** (2 μM) and (b) Lifetime at λ_{ex}= 515 nm in PBS buffer (pH= 7.4) 1.Ca^{2+} (500 mM); 2. Cu^{2+} (100 mM); 3. K^{+} (150 mM); 4. Mg^{2+} (2 mM); 5. Fe^{2+} (100 mM); 6. Zn^{2+} (100 mM); 7. Cysteine (Cys, 1 mM); 8. Alanine (Ala, 1 mM); 9. Lysine (Lys, 1 mM); 10. Glucose (10 mM); 11. Glutathione (GSH, 5 mM); 12. Human Serum Albumin (HSA, 200 mM); 13. H_2O_2 (100 mM); 14. ·OH (10 mM H_2O_2 + 10 mM Fe^{2+}–EDTA); 15. Glycerol.

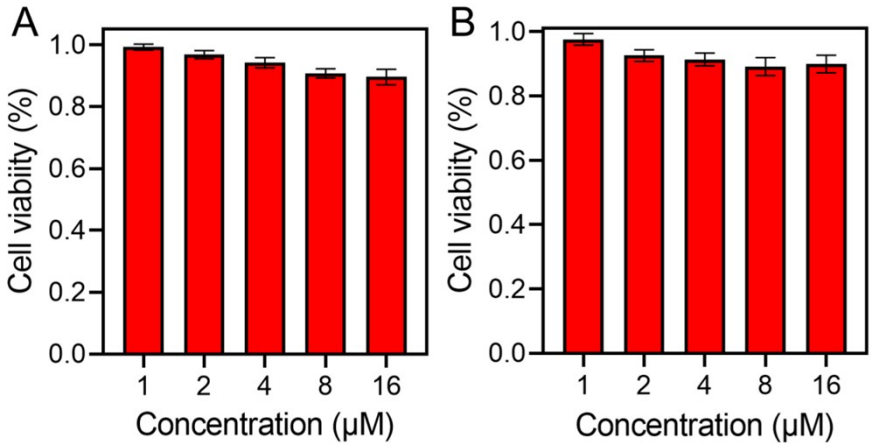


Fig. S5 (A) Cytotoxicity date of **GA-Vis** (LO2 cells incubated for 24 h). (B) Cytotoxicity date of **GA-Vis** (HepG2 cells incubated for 24 h).

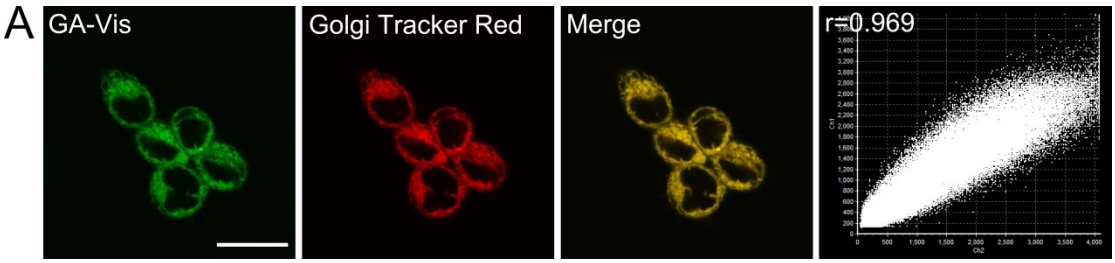


Fig. S6 (A) Colocalization fluorescence images of HEK-293 cells incubated with **GA-Vis** (2 μM) for 30 min, and then with Golgi Tracker Red (1 μM) for 30 min. Scale bars: 20 μm.

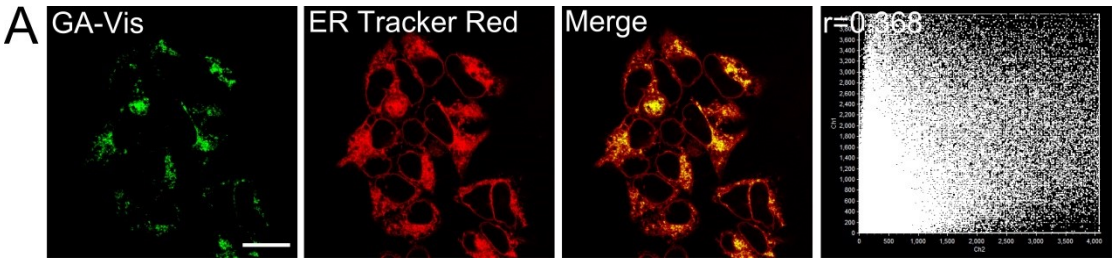


Fig. S7 (A) Colocalization fluorescence images of HEK-293 cells incubated with **GA-Vis** (2 μM) for 30 min, and then with ER Tracker Red (1 μM) for 30 min. Scale bars: 20 μm.

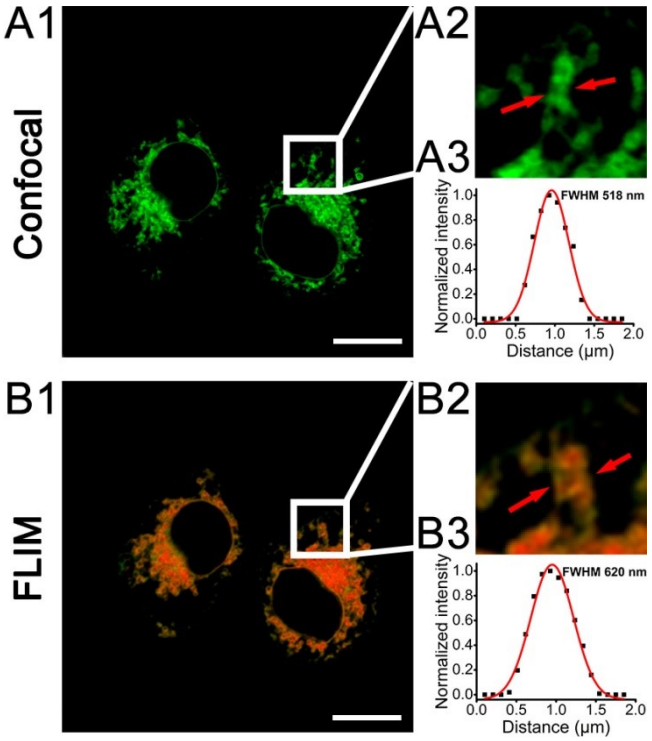


Fig. S8 Fluorescence and lifetime imaging of **GA-Vis** in LO2 cells. (A1) Fluorescence imaging of **GA-Vis** (2 μM) incubated with LO2 cells for 30 min. (A2) the magnified image of the region of interest (ROI) in (A1). (A3) Intensity profiles of the red arrow marked regions in (A2). (B1) FLIM of **GA-Vis** incubated with HeLa cells for 30 min. (B2) the magnified image of the region of interest (ROI) in (B1). (B3) Intensity profiles of the red arrow marked regions in (B2). Data were fitted with the Gaussian function. Scale bars: 20 μm.

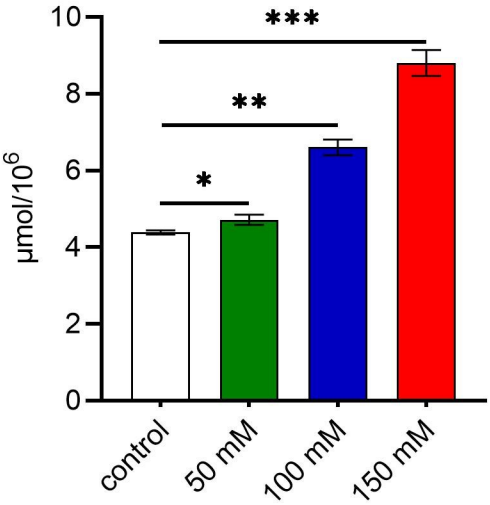


Fig. S9 The level of triglyceride of cells under different incubation conditions after 24 h. The results are presented as mean ± standard deviation (n = 3). Significant differences (N.S.: no significant difference; *p < 0.05; **p < 0.01; ***p < 0.001) are performed by Student's t-test.

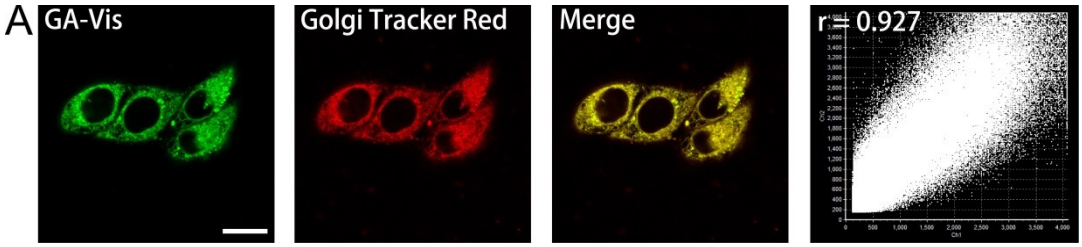


Fig. S10 (A) Colocolization fluorescence images of LO2 cells treatment with ethanol (100 mM) after 72 h, firstly stain with **GA-Vis** (2 μM) for 30 min and then with Golgi Tracker Red (1 μM) for 30 min. Scale bar: 20 μm.

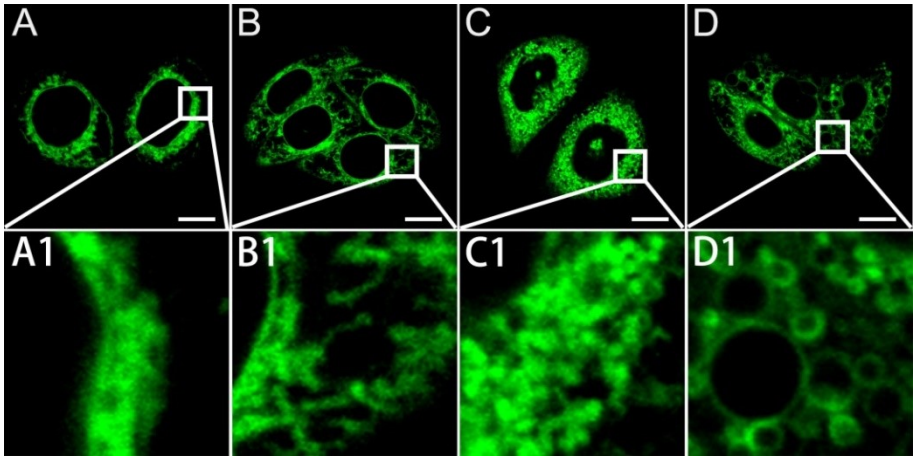


Fig. S11 (A-D) Fluorescence images of Golgi in Fig. 4. Scale bar: 20 μm.

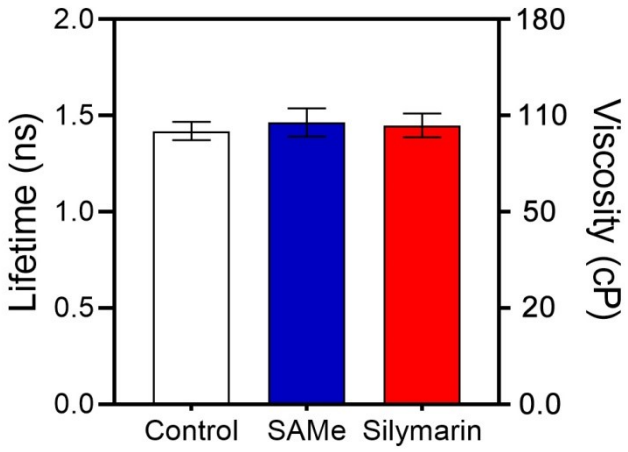


Fig. S12 Average lifetime and correspond viscosity of cells in Golgi after 72 h under different culture conditions. The results are presented as mean ± standard deviation (n = 3).

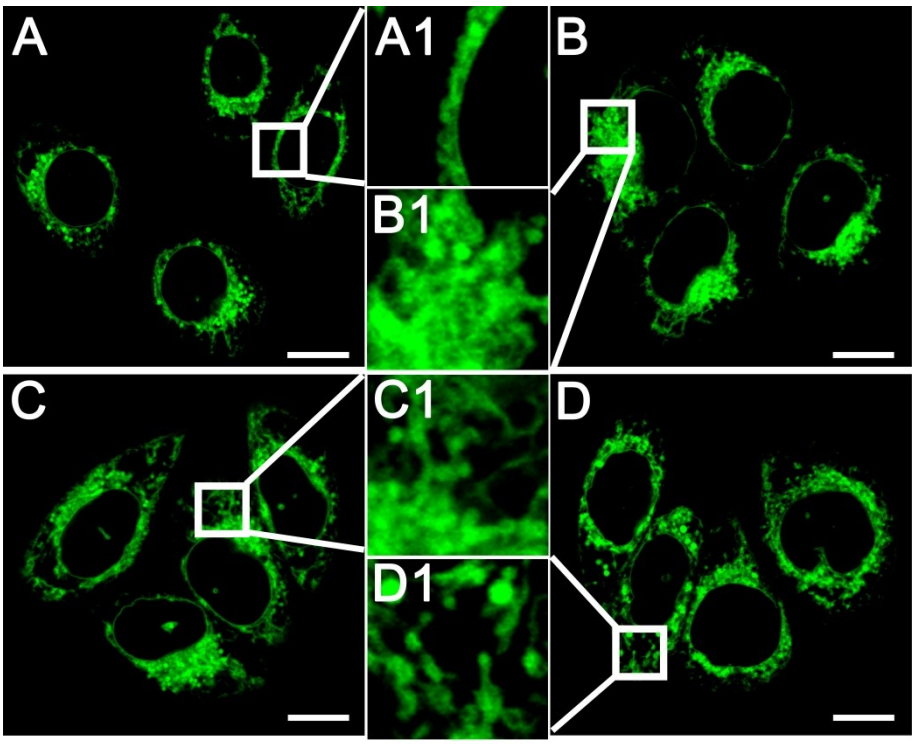


Fig. S13 (A-D) Corresponding fluorescence imaging in Fig. 5; (A1-D1) The magnified image of the region of interest in (A-D), respectively. Scale bar: 20 μm.

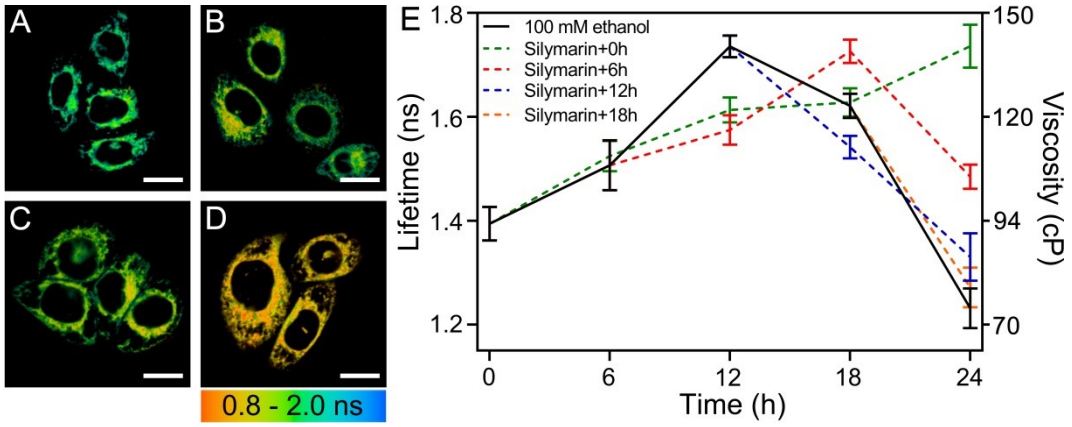


Fig. S14 FLIM imaging of Golgi in LO2 cells treatment with silymarin at different time under ethanol stimulation conditions after 24 h. (A-D) FLIM imaging of LO2 cells treatment with silymarin at 0, 6, 12, 18 h, respectively. (A1-D1) The magnified image of the region of interest in (A-D), respectively. (E) Changes of Golgi viscosity correspond to A-D. The results are presented as mean ± standard deviation (n = 3). Scale bar: 20 μm.

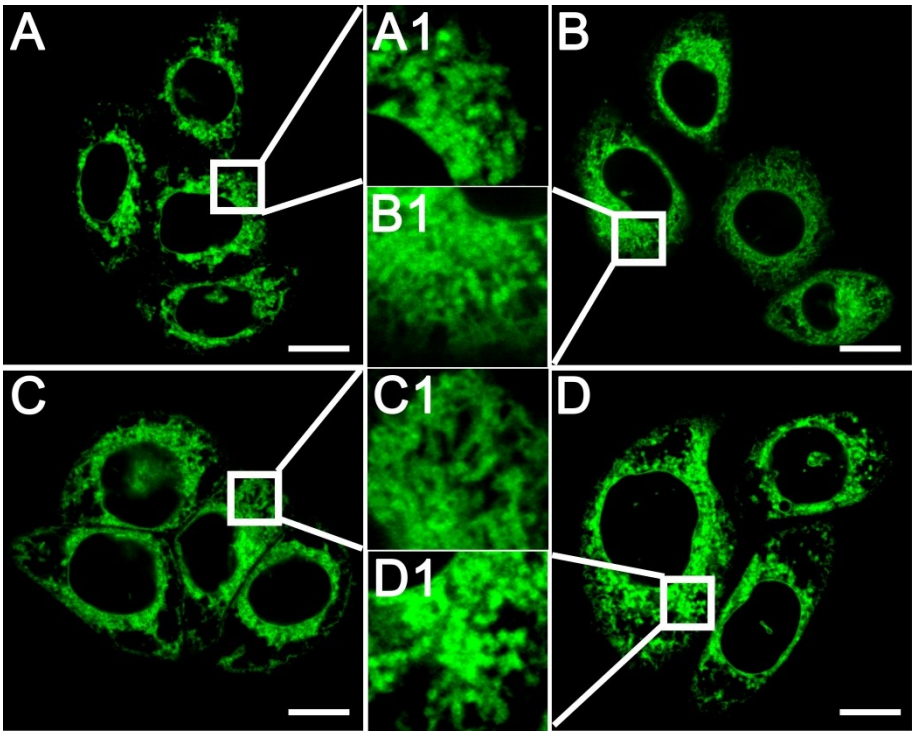


Fig. S15 (A-D) Corresponding fluorescence imaging in Fig. S11; (A1-D1) The magnified image of the region of interest in (A-D), respectively. Scale bar: 20 μm.

3. Characterization of spectra

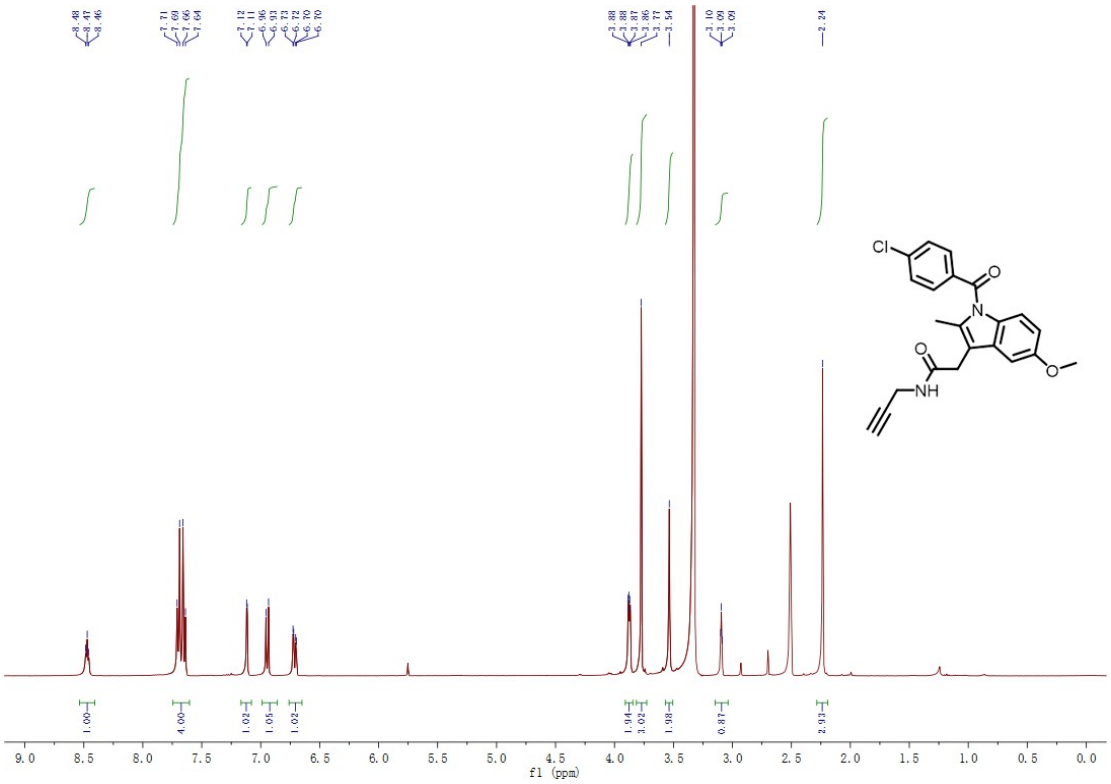


Figure S11. The NMR for IMC-1.

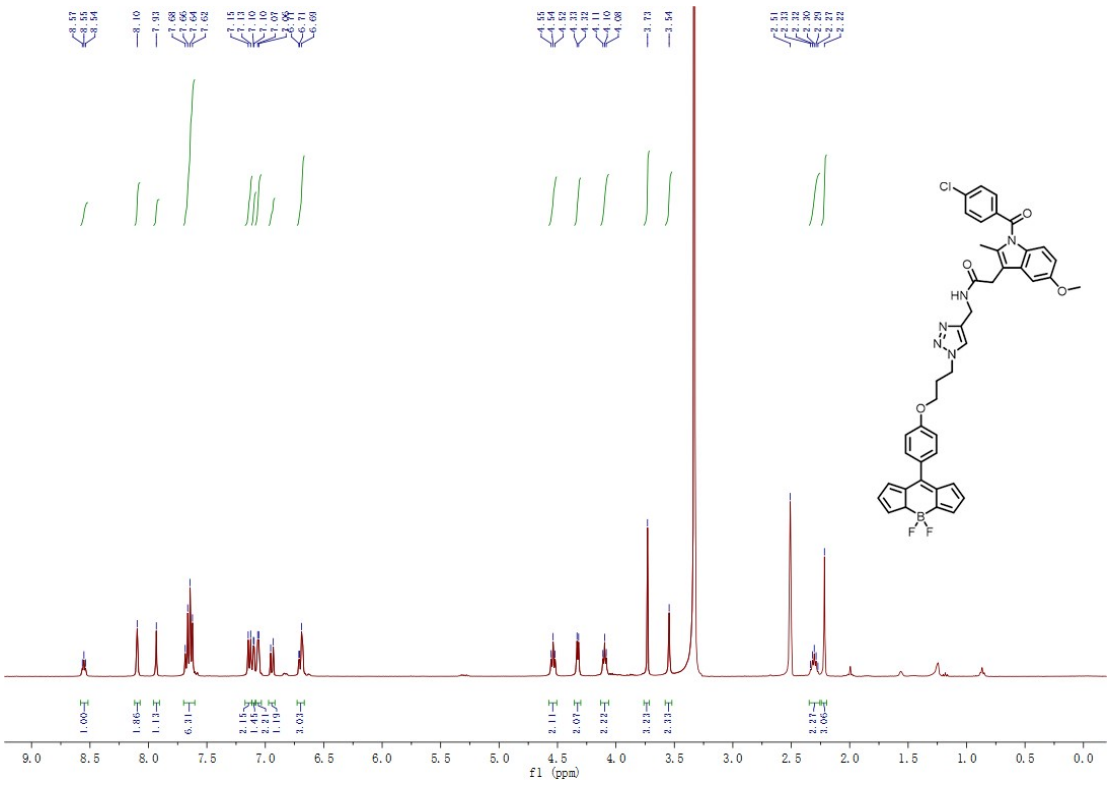


Figure S12. The NMR for **GA-Vis**.

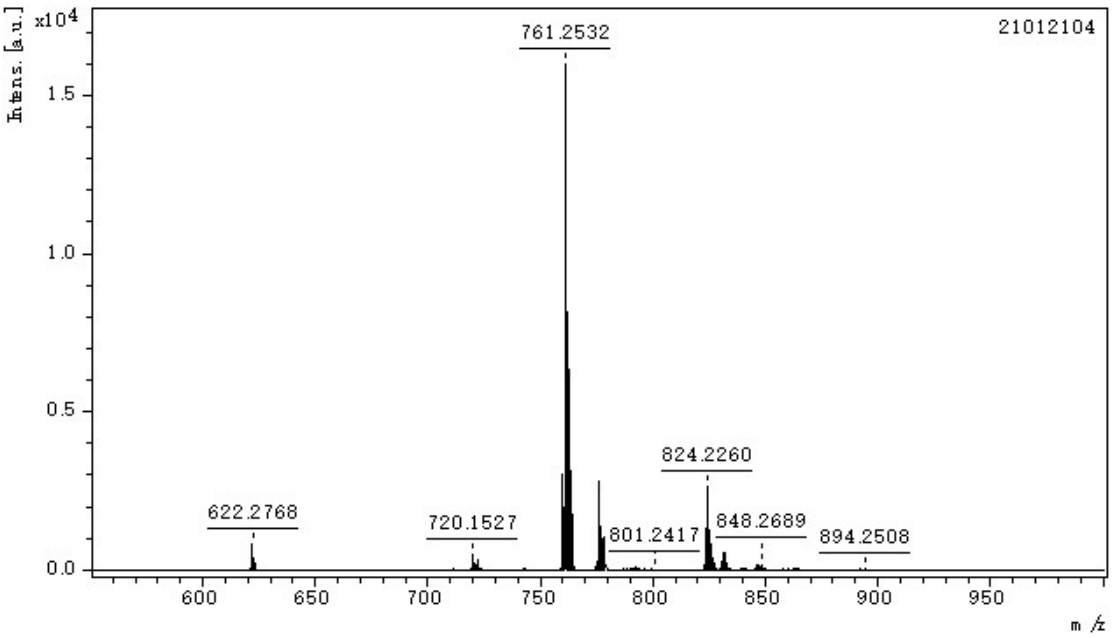


Figure S13. The HRMS for **GA-Vis**.

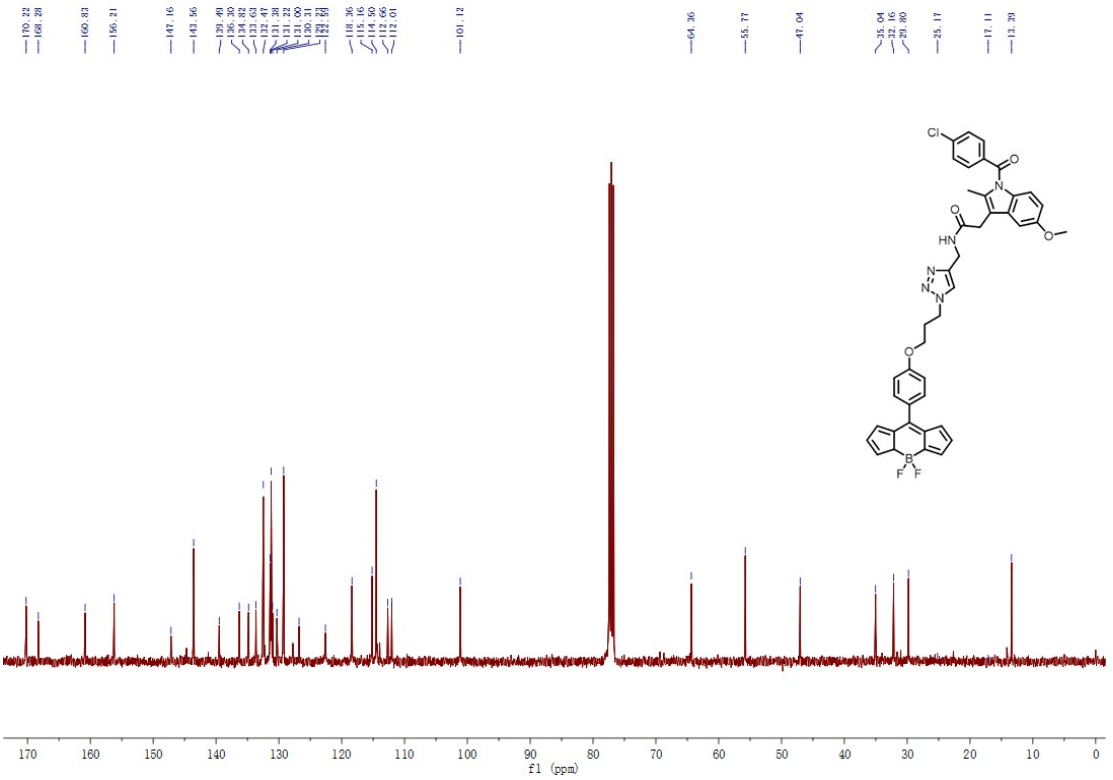


Figure S14. The NMR for **GA-Vis**.

4. References

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(2) Poh, J. S.; Makai, S.; von Keutz, T.; Tran, D. N.; Battilocchio, C.; Pasau, P.; Ley, S. V. *Angew. Chem. Int. Ed.* 2017, ***56*,** 1864-1868.