Electronic Supplementary Information for

Golgi-targeted off-on fluorescent probe for real-time monitoring pH changes in vivo

Li Fan[*†a], *Xiaodong Wang*[†a], *Jinyin Ge*[a], *Feng Li*[*b], *Caihong Zhang*[a], *Bo Lin*[a], *Shaomin Shuang*[a] *and Chuan Dong*[*a]

[a]*Institute of Environmental Science, College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, 030006, P. R. China.*
[b]*Department of Chemistry, Centre for Biotechnology, Brock University, St. Catharines, Ontario, Canada, L2S 3A1*

E-mail: fanli128@sxu.edu.cn; **fli@brocku.ca**; **dc@sxu.edu.cn.**

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Experimental section

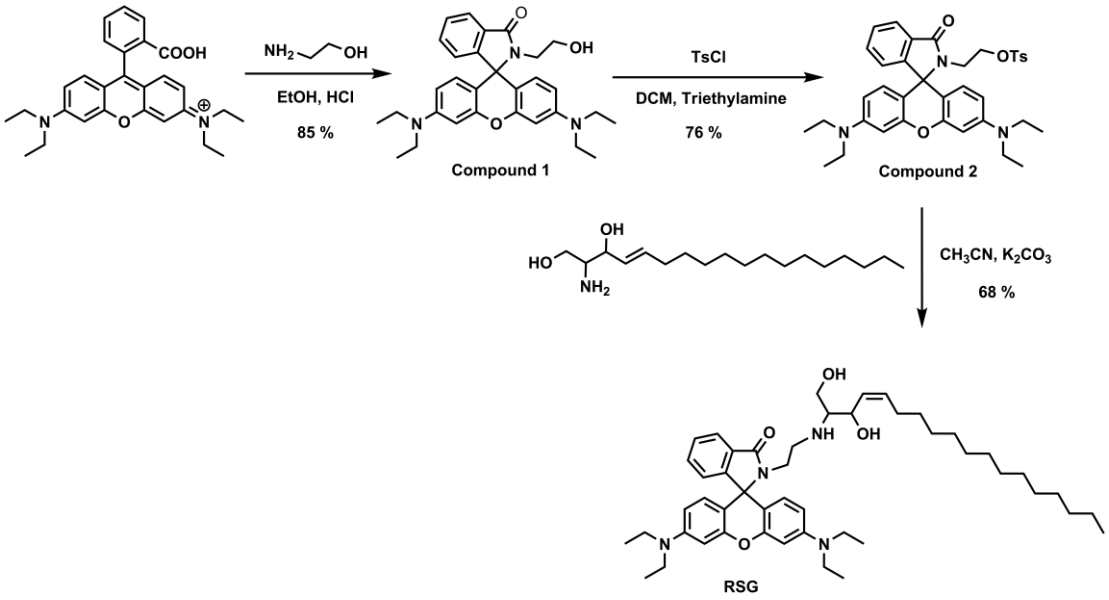
Materials and general instruments

All chemicals were commercially available and were of analytical grade. Rhodamine B, 2-aminoethanol ethyl iodide, trimethylamine, TsCl, sphingosine, cell counting kit-8 (*CCK-8*), N-ethylmaleimide (NEM), N-acetylcysteine (NAC), dexamethasone (DEX) and lipopolysaccharides (LPS) were obtained from Sigma-Aldrich. Nigericin (sodium salt) were commercially available from J&K Chemical Co., Ltd. NBD C6-Ceramide, LysoTracker Green DND-26 and Mito Tracker Green were purchased from Invitrogen (USA). Bafilomycin A1, Carbonyl cyanide m-chlorophenyl hydrazine (CCCP) and chloroquine were purchased from MedChemExpress (China). $^1O_2$ was prepared according to the reported procedures.[1] All other chemicals were bought from Beijing Chemical Regent Co., Ltd.

NMR spectra were acquired with a Bruker instrument (BrukerBioSpin AG Ltd., Beijing, China) with TMS as the internal standard in DMSO-d_6 or $CDCl_3$ of 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR, respectively. High resolution mass spectrometric data were determined on an Agilent Accurate-Mass-Q-TOF MS 6520 system equipped with an electrospray ionization (ESI) source (Agilent, USA). Deionized water was prepared on a Milli-Q water purification system (Millipore). pH values were obtained on a Beckman Φ50 pH meter (Shanghai INESA Scientific Instrument Co., Ltd. Shanghai, China). The UV-visible spectra measurements were taken on a TU-1901 UV-visible double-beam spectrometer (Beijing Purkinje General Instrument Co., LTD, Beijing, China). Fluorescence spectra measurements were recorded on a FLS-920 Full-featured Fluorescence Spectrometer (Edinburgh Instruments, EI) equipped with a xenon discharge lamp using 1 ml Fluor Micro Cell.

Synthesis and characterization

Synthesis routine of RSG



Scheme **S1** The synthetic routine of **RSG**

Synthesis of Compound 1. A mixture of 1.00 g (2.08 mmol) Rhodamine B, 25 ml 2-aminoethanol ethyl iodide and 15 mL (10 mM) hydrochloric acid was refluxed in 50 mL anhydrous methanol for 48 h until the deep-red color of rhodamine B changed to deep green. The mixture was dried over anhydrous magnesium sulfate, and then concentrated under vacuum. The residue was purified by chromatography on a silica gel column using dichloromethane/methanol (20:1, v/v) as the mobile phase, affording compound **1** as a light pink powder 0.86 g (85 % yield). ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.18-1.20 (m, 12H, -CH_3), 3.31-3.37 (m, 9H, -CH_2-), 3.49 (s, 2H, -CH_2-), 4.21 (s, 1H, -CH_2-), 5.32 (s, 1H, -OH), 6.31-6.32 (m, 2H, Ar-H), 6.40 (M, 2H, Ar-H), 6.51-6.52 (m, 2H, Ar-H), 7.09 (s, 1H, Ar-H), 7.47 (s, 2H, Ar-H), 7.92 (s, 1H, Ar-H).

Synthesis of Compound 2. A mixture of 0.50 g (1.03 mmol) compound 1, 1.0 g (9.89 mmol) trimethylamine and 0.25 g (1.31 mmol) TsCl was refluxed in 100 mL dichloromethane under nitrogen overnight. The mixture was dried over anhydrous magnesium sulfate, and then concentrated under vacuum. The residue was purified by chromatography on a silica gel column using petroleum ether/ethyl acetate (2:1,

v/v) as the mobile phase, affording compound **2** as a white powder 0.50 g (76 % yield). ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 1.19 (t, 12H, -CH_3), 2.43 (s, 3H, -CH_3), 3.36 (m, 10H, -CH_2-), 3.75 (s, 2H, -CH_2-), 6.26 (d, 2H, -CH-), 6.38-6.41 (m, 4H, -CH-), 7.09 (s, 1H, -CH-), 7.28 (s, 2H, -CH-), 7.43 (s, 2H, -CH-), 7.64 (d, 2H, -CH-), 7.88 (s, 1H, -CH-).

Synthesis of Compound 3. A mixture of 0.06 g (0.08 mmol) compound 2 in 30 mL anhydrous acetonitrile, 0.02 g (0.07 mmol) sphingosine in 30 mL anhydrous dichloromethane and 0.009 g (0.07 mmol) anhydrous potassium carbonate was refluxed under nitrogen for 12 h. The mixture was dried over anhydrous magnesium sulfate, and then concentrated under vacuum. The residue was purified by chromatography on a silica gel column using dichloromethane as the mobile phase, affording **RSG** as a light pink powder 0.04 g (68 % yield). ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 0.90 (m, 3H, -CH_3), 1.16 (m, 12H, -CH_3), 1.17-1.30 (m, 22H, -CH_2-), 1.91 (m, 1H, -CH-), 2.04 (m, 2H, -CH_2-), 2.20 (1H, -OH), 2.95 (s, H, -CH_2-), 3.40 (m, 8H, -CH_2-), 3.52 (m, 1H, -CH_2-), 3.56 (d, 1H, -CH_2-), 4.00-4.18 (m, 3H, -CH_2-), 4.34-4.41 (m, 1H, -CH-), 5.12 (m, 1H, -CH=), 5.35 (m, 1H, -CH=), 6.41-6.56 (d, 5H, Ar-H), 7.20 (m, 1H, Ar-H), 7.67 (m, 2H, Ar-H), 8.17-8.18 (d, 1H, Ar-H), 8.40-8.41 (d, 1H, Ar-H). ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm): 11.52,13.06,19.91, 22.35, 25.53, 26.71, 29.37, 39.78, 43.97, 51.09, 57.98, 60.84, 62.70, 70.32, 78.70, 97.62, 100.88, 103.17, 108.25, 114.78, 121.11, 124.82, 128.43, 132.50, 140.22, 141.80, 149.33, 153.49, 155.12, 160.34, 166.76, 177.81. HR-MS m/z: $[M+H]^+$ calculated for $C48H70N4O4^+$, 767.5470; measured, 767.5460.

Synthesis of CPM. A mixture of 1.00 g (4.32 mmol) compound **2**, 0.83 g (4.44 mmol) compound **3** and 0.53 ml piperidine are stirred in 15 ml absolute ethanol at room temperature for 10 min, followed at 65 ℃ over night. Then the reaction was cooled to room temperature, and the precipitated solid was collected by filtration. Then the red power (1.50 g, 3.71 mmol) was obtained after the precipitate washed with absolute ethanol and dried. Yield: 86 %. ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 8.57 (s, 1H, Ar-H), 8.05-8.00 (d, 1H, Ar-H), 7.83-7.78 (d, 1H, Ar-H), 7.65-7.62 (m, 3H, Ar-H), 6.67-6.64 (m, 3H, Ar-H), 6.51-6.50 (m, 1H, Ar-H), 3.93-390 (m, 4H, -CH_2-),

3.31-3.29 (m, 4H, -CH_2-), 3.13 (s, 3H, -CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm): 186.36, 160.87, 158.17, 154.71, 152.60, 148.56, 143.82, 131.38, 130.48, 126.46, 121.52, 114.57, 109.94, 108.99, 97.10, 66.70, 48.06, 40.30. HR-MS m/z: $[M+H]^+$ calculated for $C_{24}H_{24}N_2O_4^+$, 405.1809; measured, 405.1805.

UV-vis and fluorescence titrations

Doubly distilled water was used to prepare all the aqueous solutions. **RSG** was dissolved in DMSO to obtain 1.0 mM stock solution. The solution with final concentration of 50 μM for UV-vis spectroscopic determination was obtained by diluting the stock solution to Britton-Robinson (B-R) buffer solution (40 mM, acetic acid, boric acid, and phosphoric acid) containing 10 % DMSO at various pH values. The solution with final concentration of 10 μM for fluorescence spectroscopic determination was obtained by diluting the stock solution B-R buffer solution containing 1 % DMSO at various pH values. Spectral data were recorded after 10 min for equilibrating each addition. The fluorescence spectra were monitored with the excitation and emission slit width both of 2 nm, and the excitation wavelength of 570 nm. All spectroscopic experiments were performed at room temperature.

Cell cytotoxicity assay

In order to evaluate the cell cytotoxicity of **RSG**, a standard *CCK-8* assay[2] (cell counting kit-8) in live SMMC-7721 cells was performed. About 10000 cells/well in 200 μL cell culture medium were seeded in 96-well microplate and cultured overnight at 37 ℃ in a 5 % CO_2 atmosphere. Then the medium was replaced with fresh medium that containing **RSG** at various final concentrations of 0.5 μM, 1 μM, 10 μM, 20 μM and 50 μM for 24 h, respectively. After washing the cells with fresh medium three times, 20 μL of *CCK-8* mixed in 180 μL PBS was introduced into each well of the 96-well microplate for another 4 h. Then the absorbance was measured at 450 nm with an ELISA microplate reader. The cell viability was expressed as relative to the control cells taken as 100 % metabolic activity.

Cell culture and fluorescence imaging

SMMC-7721, BEAS-2B, LO2, HIEC cells, A549, and HIC cells were cultured in

RPMI1640 or DEME medium supplemented with 10 % fetal bovine serum 37 ℃ in a 5 % CO_2 atmosphere. For live cell imaging, **RSG** was added to the cultured cells in a glass-bottomed dish for 10 min and washed with 10 mM PBS (pH 7.4) three times. After replacement of the medium, cells were imaged using a confocal laser scanning microscope (Zeiss, LSM880) with a 63 × oil-immersion objective lens. The fluorescence images were carried out on a confocal laser scanning microscope (Zeiss, LSM880) with red channel (Ex = 561 nm, Em = 568 - 650 nm) for **RSG**.

For colocalization experiments

SMMC-7721 cells were passed and plated on glass-bottomed dishes at a density about 1×10^5 for 24 h. Then 5 μM NBD, 10 μM LysoTracker Green DND-26 or 0.2 μM Mito Tracker Green was added to 1 mL cells medium, respectively. After 30 min, 10 μL **RSG** (1 mM in DMSO) at 10 μM final concentrations was added and co-incubated for additional 10 min. The fluorescence images were carried out on a confocal laser scanning microscope (Zeiss, LSM880) with red channel (Ex = 561 nm, Em = 568 - 650 nm) for **RSG**, Green channel (Ex = 488 nm, 493 - 550 nm) for NBD, Lyso Tracker DND-26 or Mito Tracker Green, respectively.

For intracellular pH calibration using RSG

SMMC-7721 cells were pretreated with media containing **RSG** (10 μM) and NBD (5 μM) for 30 min at 37 °C. Then the cells were washed with PBS (pH 7.4) for three times, and replaced with 10 μg/mL nigericin in high K^+ buffer (including 120 mM KCl, 30 mM NaCl, 0.5 mM $MgSO_4$, 1 mM $CaCl_2$, 20 mM HEPES, 20 mM NaOAc, 1 mM NaH_2PO_4 and 5mM glucose) [41] at various pH (3.00, 4.00, 5.00, 6.00 and 7.40), respectively. After 10 min, the red (**RSG**) and green (NBD) channel images were recorded with a confocal laser scanning microscope. A pH calibration curve (F_{red} / F_{green} *vs* pH) was then constructed.

For real-time monitoring the stimulated Golgi pH changes in live cells.

SMMC-7721 cells were pretreated with media containing **RSG** (10 μM) and NBD (5 μM) for 30 min at 37 °C. Then the cells were washed with PBS (pH 7.4) for three

times, and incubated with different drugs such as 100 nM bafilomycin A1, 2 μM CCCP, 30 mM NH_4Cl, 50 μM chloroquine, 100 μM H_2O_2, 100 μM NEM, 100 μM NAC or 100 μM NaClO for another 5-20 min, respectively.

Monitoring pH changes induced by LPS *in vivo*.

BALB/c mice (20-25 g) were purchased from Laboratory Animals Center of Shanxi Medical University (Taiyuan, China) for *in vivo* imaging. All animal experiments were performed in compliance with the Animal Management Rules of the Ministry of Health of the People's Republic of China (Document no. 55, 2001) and approved by the Animal Care and Use Committee of Shanxi University. We have taken great efforts to reduce the number of animal used in these studies and also taken effort to reduce animal suffering from pain and discomfort.

BALB/c mice were given an intraperitoneal injection of LPS (200 μL in saline, 1.0 mg/mL). Then after 6 h, the mice were intraperitoneally injected with **RSG** (200 μL, 50 μM) for another 20 min. Meanwhile, mice with only LPS (200 μL in saline, 1.0 mg/mL) treated for 6 h and mice with only **RSG** (200 μL, 50 μM) treated for 20 min were prepared, respectively. Before imaging, the mice were anesthetized by an intraperitoneal injection of 4 % chloral hydrate (200 μL) for 5 min. *In vivo* images were then taken by using a Bruker small animal *in vivo* Xtreme imaging system (Ex = 570 nm, Em = 600 nm).

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Table S1 Performances of the representative Golgi-targetable optical probes and RSG designed herein

Probe	$\lambda_{abs}/\lambda_{em}$ (nm)	Dye platform	Targetable group	Pearson's colocalization coefficient	Application	Ref.
NBD C6-ceramide	466/536	Benzoxadiazol	Sphingosine	—	Commercial Golgi Green dye	S1
BODIPY FL C5-ceramide	505/511	BODIPY	Sphingosine	—	Commercial Golgi Green dye	S2
BODIPY TR C5-ceramide	589/617	BODIPY	Sphingosine	—	Commercial Golgi Red dye	S3
LC-CQDs	350/420	Chiral carbon quantum dots	L-Cysteine	> 0.90	Targeting and long-term *in situ* Golgi imaging in live cells	S4
SZnC	388/499	Naphthalene	—	0.92	Real-time monitoring Golgi Zn^{2+} change in live cells and tissues	S5
ANQ-IMC-6	457/547	Quinoxaline	—	0.97	Monitoring the COX-2 change in cancer cells and tissues	S6
Niblue-C6-IMC	630/670	Nile blue	—	> 0.93	Visualizing tumor sites in cancer cells and mouse tumor model	S7
Cer-SiR	650/659	SiR-OH	Sphingosine	0.73	Super-Resolution imaging Golgi in live cells	S8
Gol-SiRhoNox	630/665	6-carboxy-SiR	Myristoyl	—	Monitoring the Golgi Fe^{2+} changes in living cells	S9
Yb–L[2]	430/(657/720)	Porphyrin-cyclen	—	—	Golgi apparatus imaging and photodynamic therapy	S10
Golgi-P	700/(800/825)	Merocyanine	L-Cysteine	0.93	Visualizing the Golgi polarity in live cells and live brains of mice with depression-like behaviors	S11
RSG	570/600	Rhodamine B	Sphingosine	> 0.90	Real-time monitoring Golgi pH in live cells and LPS-mediated mouse	This work

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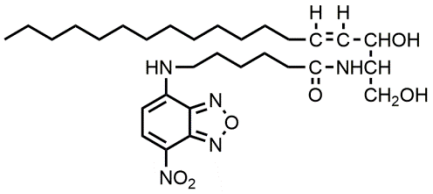

NBD C6-ceramide

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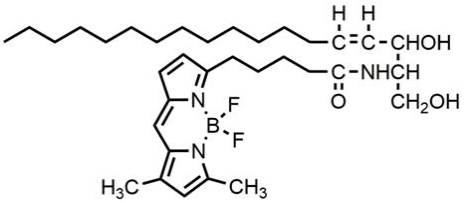
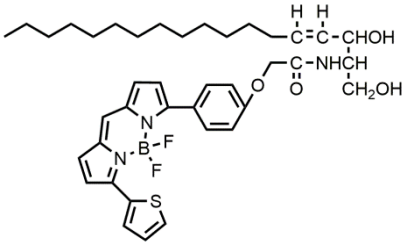


BODIPY FL C5-ceramide

J. Cell. Biol., 1991, 113, 1267

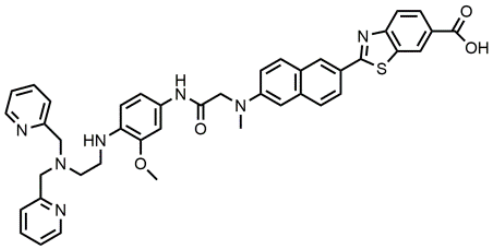


BODIPY TR C5-ceramide

Mol. Biol. Cell., 1995, 6, 135

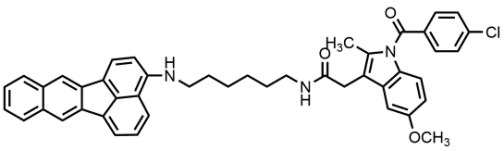
LC-CQDs

Chem. Sci., 2017, 8, 6829



SZnC

Chem. Commun., 2015, 51, 12099



ANQ-IMC-6

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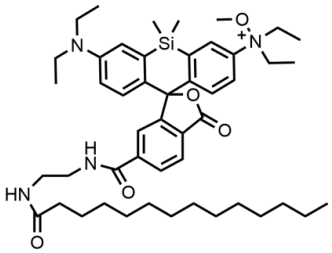
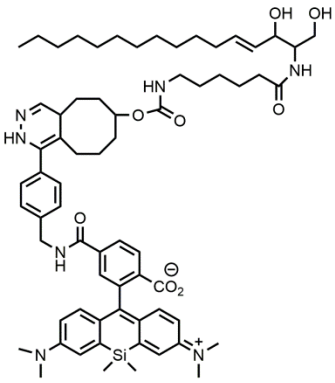


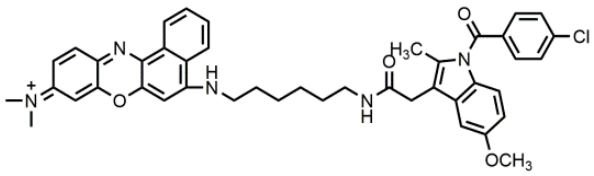
Gol-SiRhoNox

Chem. Sci., 2019, 10, 1514

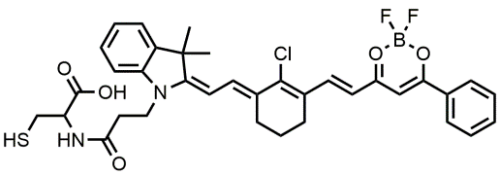


Cer-SiR

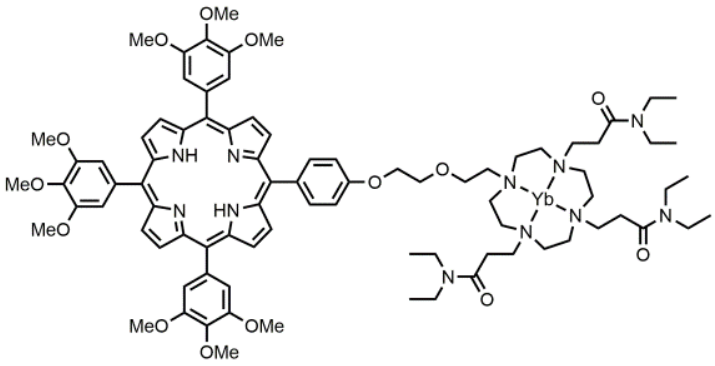
Angew. Chem. Int. Ed., 2014, 53, 10242



Niblue-C6-IMC
Chem. Commun., 2015, 51, 792



Golgi-P
Anal. Chem., 2019, 91, 3382



Yb–L^2
Chem. Commun., 2012, 48, 9646

Fig. S1 Chemical structures of representative Golgi-targetable optical probes listed in **Table S1**.

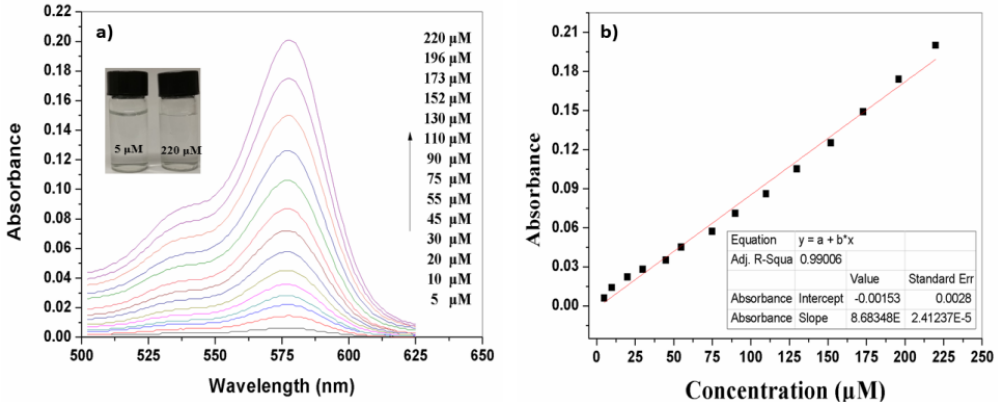


Fig. S2. (a) Changes of the absorption spectra of **RSG** with increasing the concentration from 5 μM to 220 μM in B-R buffer solution (40 mM) containing 10 % DMSO. Inset: the clarified solution with the concentration increasing. (b) The good linearity in the concentration range of 5-220 μM.

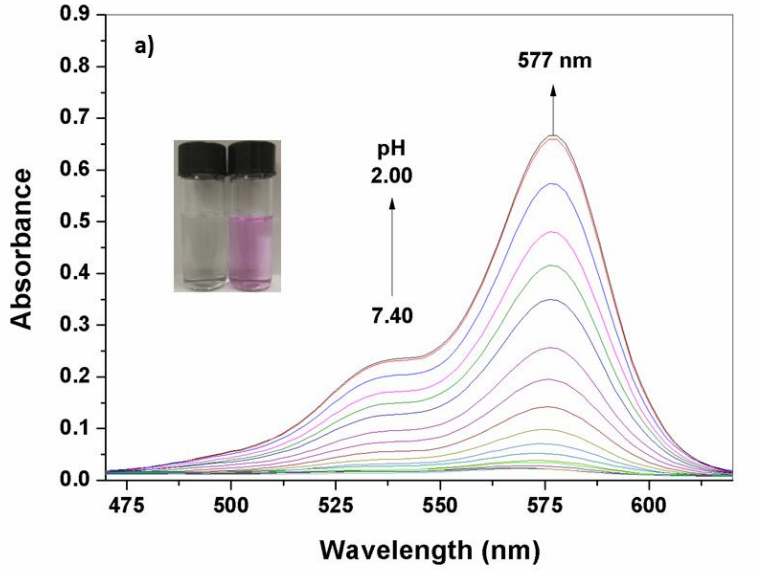


Fig. S3 Absorption spectra changes of **RSG** (50 μM) in B-R buffer solution (40 mM) containing 10 % DMSO with the pH value reducing from 7.40 to 2.00. Inset: photographs of **RSG** at different pH condition.

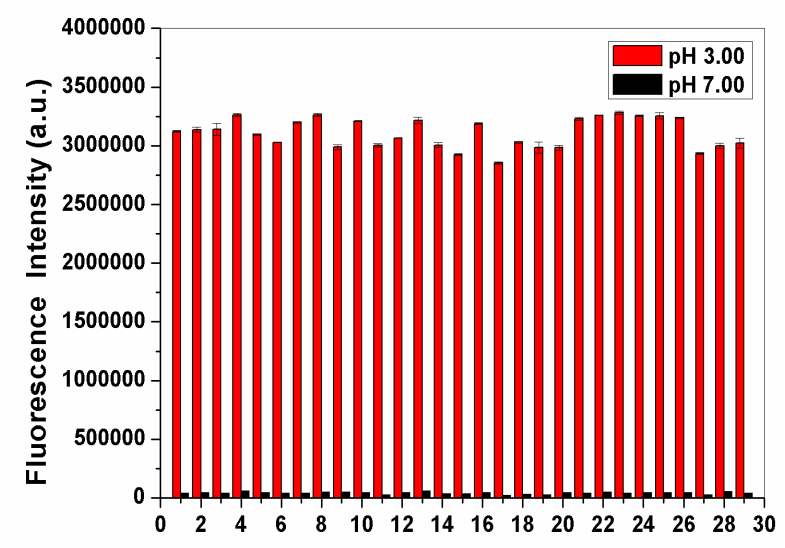


Fig. S4 Selectivity of 10 μM **RSG** to different potential interfering substances in 40 mM B-R buffer solution at pH 7.00 and 3.00, respectively: 1, blank; 2, K^+ (150 mM), 3, Na^+ (150 mM), 4, Mg^{2+} (2 mM), 5, Ca^{2+} (2 mM), 6, Ba^{2+} (0.2 mM), 7, Cu^{2+} (0.2 mM), 8, Fe^{2+} (0.2 mM), 9, Fe^{3+} (0.2 mM), 10, Ni^{2+} (0.2 mM), 11, Zn^{2+} (0.2 mM), 12, Cl^- (10 mM), 13, SO_4^{2-} (0.2 mM), 14, SO_3^{2-} (0.2 mM), 15, NO^- (0.2 mM), 16, Ac^- (0.2 mM), 17, H_2O_2 (0.1 mM), 18, ClO^- (0.1 mM), 19, $^1O_2$ (0.1 mM), 20, Cys (0.1 mM), 21, GSH (0.1 mM), 22, Hcy (0.1 mM), 23, Ala (0.1 mM), 24, His (0.1 mM), 25, Arg (0.1 mM), 26, Lys (0.1 mM), 27, Phe (0.1 mM), 28, Met (0.1 mM), 29, Leu (0.1 mM).

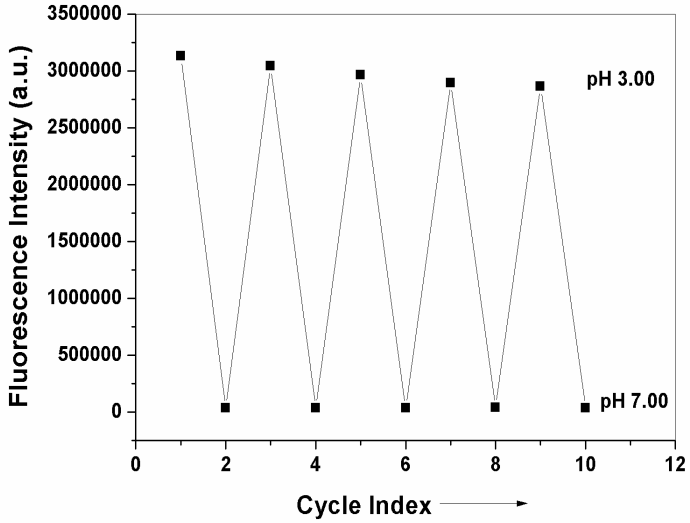


Fig. S5 Fluorescence intensity changes of **RSG** in 40 mM B-R buffer solution between pH 3.00 and 7.00. Conditions: λ_{ex} = 570 nm; λ_{em} = 600 nm.

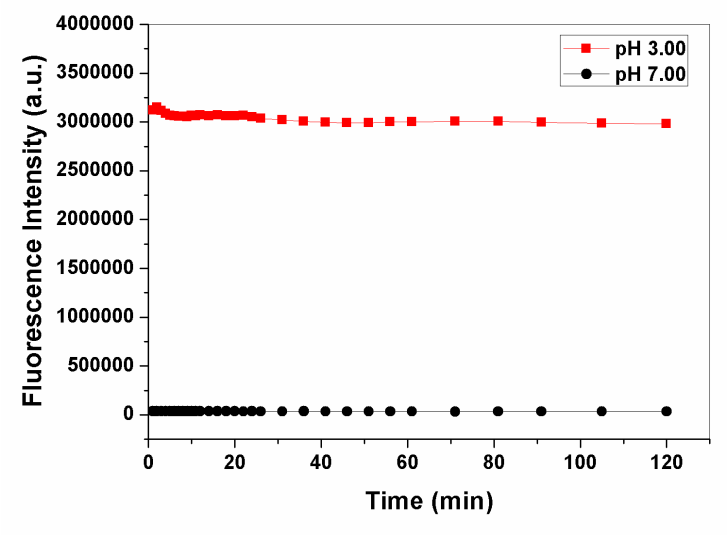


Fig. S6 Changes in fluorescence emission of **RSG** in 40 mM B-R buffer solution with times at pH 3.00 and 7.00, respectively. Conditions: λ_{ex} = 570 nm; λ_{em} = 600 nm.

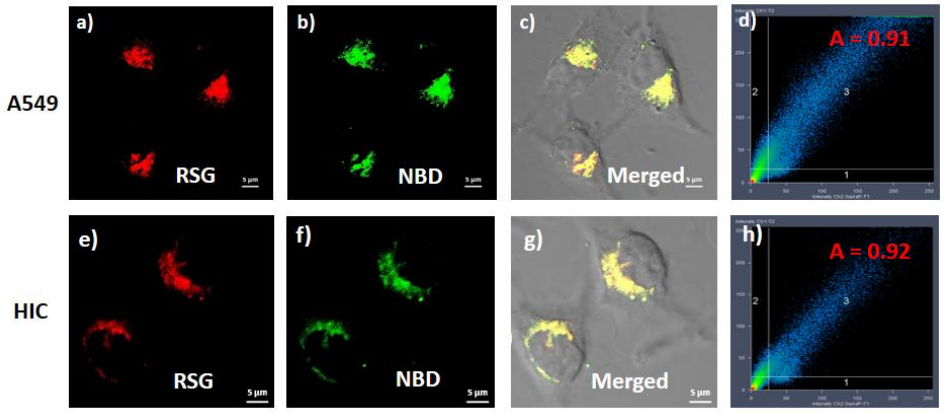


Fig. S7 Fluorescence images of 10 μM **RSG** (a, e) and 5 μM NBD (b, f) co-stained in A549 and HIC cells, respectively. (c, g) Merged images. (d, h) The Pearson's co-localization correlations of **RSG** with NBD. The red channel image was collected from 568 to 650 nm for **RSG** (λ_{ex} = 561 nm). The green channel image was collected from 493 to 550 nm for NBD (λ_{ex} = 488 nm). Scale bar: 5 μm.

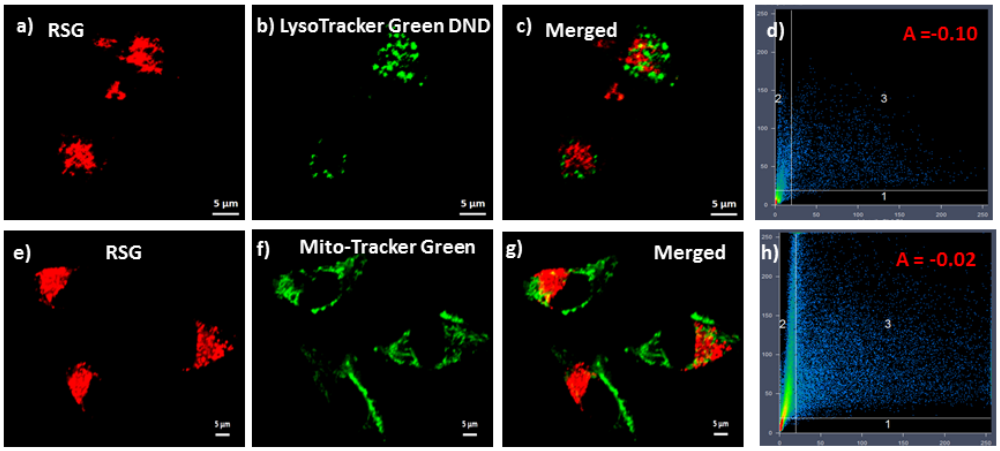


Fig. S8 Fluorescence images of 10 μM **RSG** (a, e), images of co-labeled with 10 μM LysoTracker Green DND-26 (b), 0.2 μM Mito-Tracker Green (f), merged images (c, g) in SMMC-7721 cells. (d, h) The Pearson's co-localization correlations of **RSG** with LysoTracker Green DND-26 (d, -0.10) or MitoTracker Green (h, -0.02), respectively. The red channel image was collected at 568 - 650 nm (λ_{ex} = 561 nm) for **RSG**. The green channel image was collected at 493 - 550 nm (λ_{ex} = 488 nm) for LysoTracker Green DND-26 or Mito-Tracker Green.

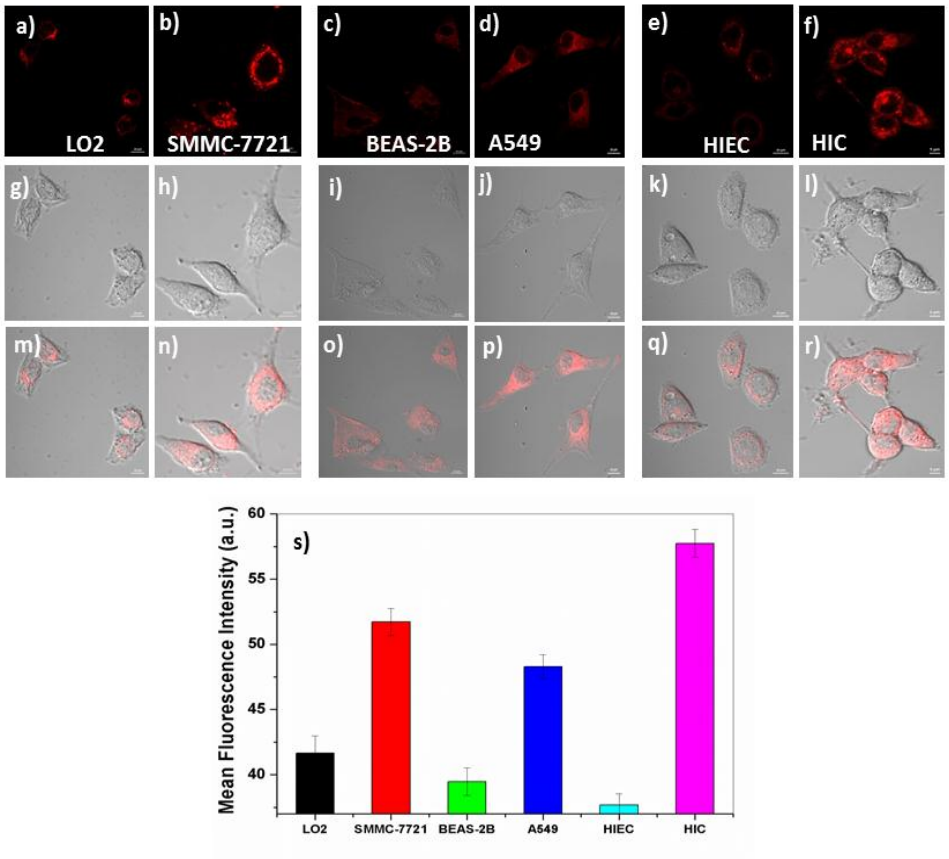


Fig. S9 Fluorescence images of 10 μM **RSG** in three types of normal cells (LO2 (a, g, m), BEAS-2B (c, i, o) and HIEC cells (e, k, q)) and their corresponding cancer cells (SMMC-7721 (b, h, n), A549 (d, j, p), and HIC cells (f, l, r)), respectively. (s) Mean fluorescence intensities of **RSG**-stained live cells. Error bars represent mean deviation (± S.D.), n = 3. The red channel image was collected at 568 - 650 nm (λ_{ex} = 561 nm) for **RSG**.

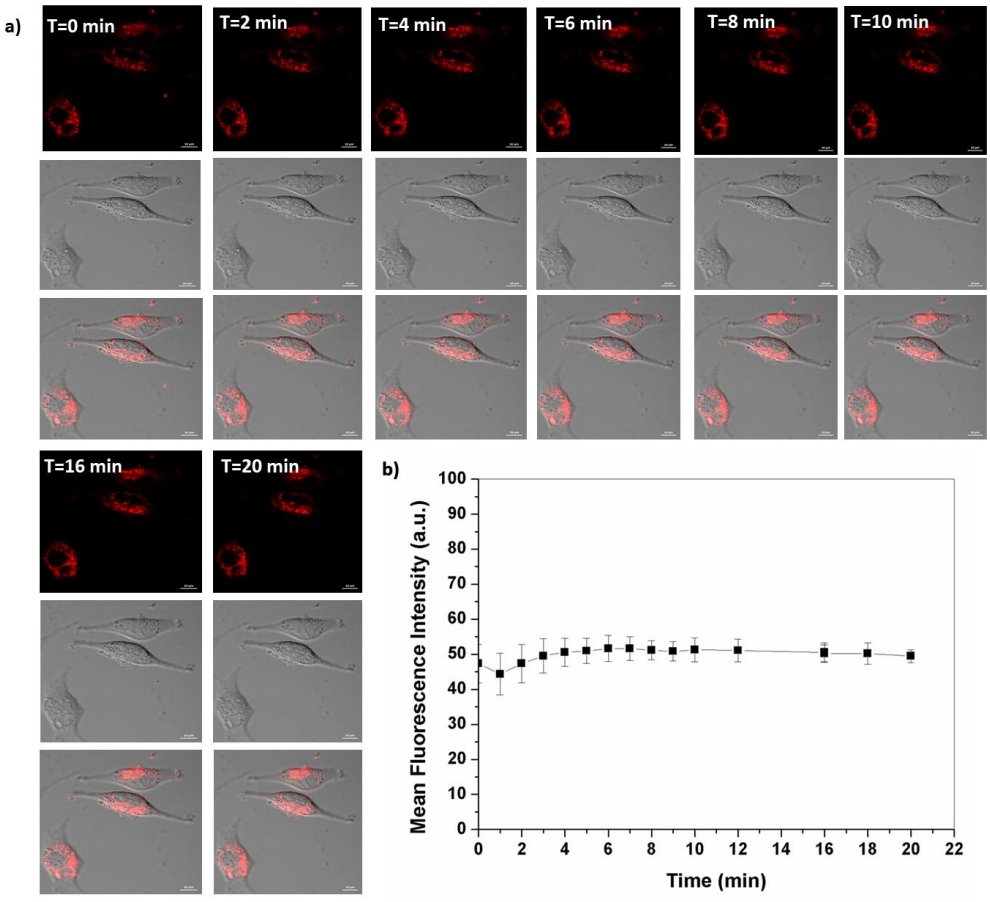


Fig. S10 (a) Fluorescence images of 10 μM **RSG** in SMMC-7721 cells during 20 min. The cells were incubated with10 μM **RSG** for 5 min at 37 ℃ and then washed with RPMI1640 medium at pH 7.4. (b) The mean fluorescence intensity of three cells. Error bars represent mean deviation (± S.D.), n = 3. The red channel image was collected at 568 - 650 nm (λ_{ex} = 561 nm) for **RSG**.

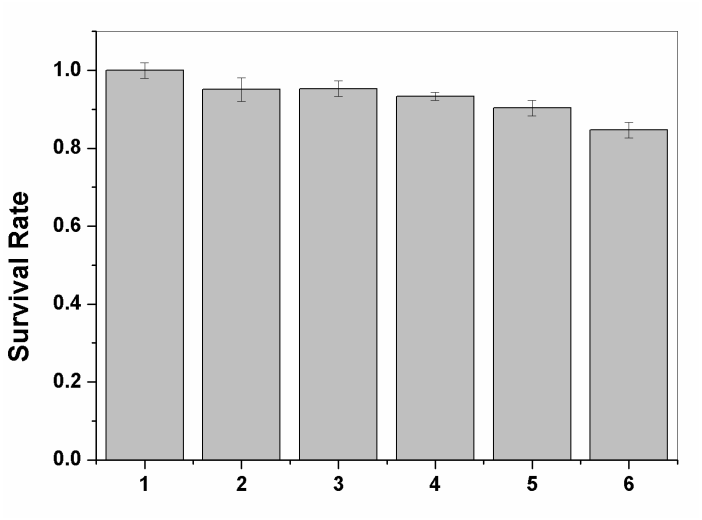


Fig. S11 Cell viability of **RSG** on SMMC-7721 cells by a standard *CCK-8* assay. 1, control; 2, 0.5 μM; 3, 1 μM; 4, 10 μM; 5, 20 μM; 6, 50 μM. Data are expressed as mean values ± standard error of the mean of three independent experiments, each performed in three triplicate.

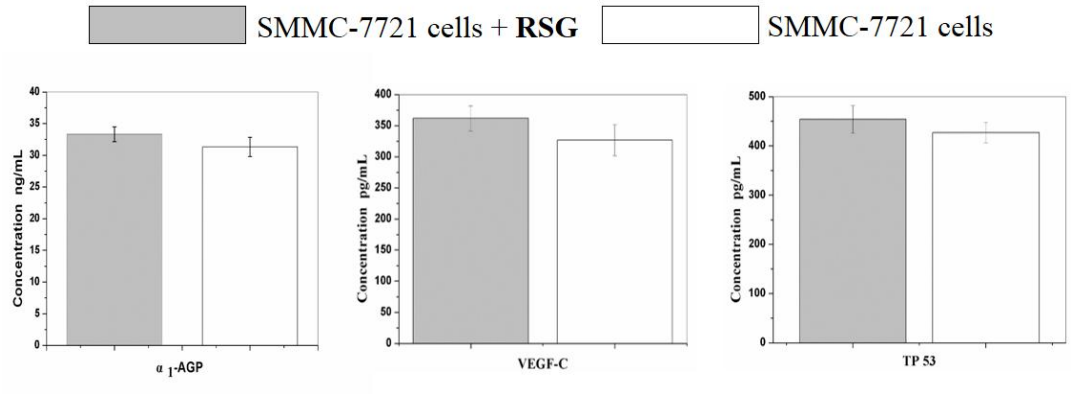


Fig. S12 Protein concentrations of SMMC-7721 cells incubated with or without **RSG**. Concentrations are quantified using commercially available ELISAs. Error bars represent mean deviation (± S.D.), n = 3. α_1-AGP: Alpha-1-Acid Glycoprotein; VEGF-C: Vascular Endothelial Growth Factor C); TP53: Tumor Protein p53.

Note: The results suggest that the quantity of glycoprotein (α_1-AGP), secreted protein (VEGF-C) and apoptosis protein (TP 53) were not affected when cells are treatment with **RSG** (10 μM), indicating the good biocompatibility of **RSG**.

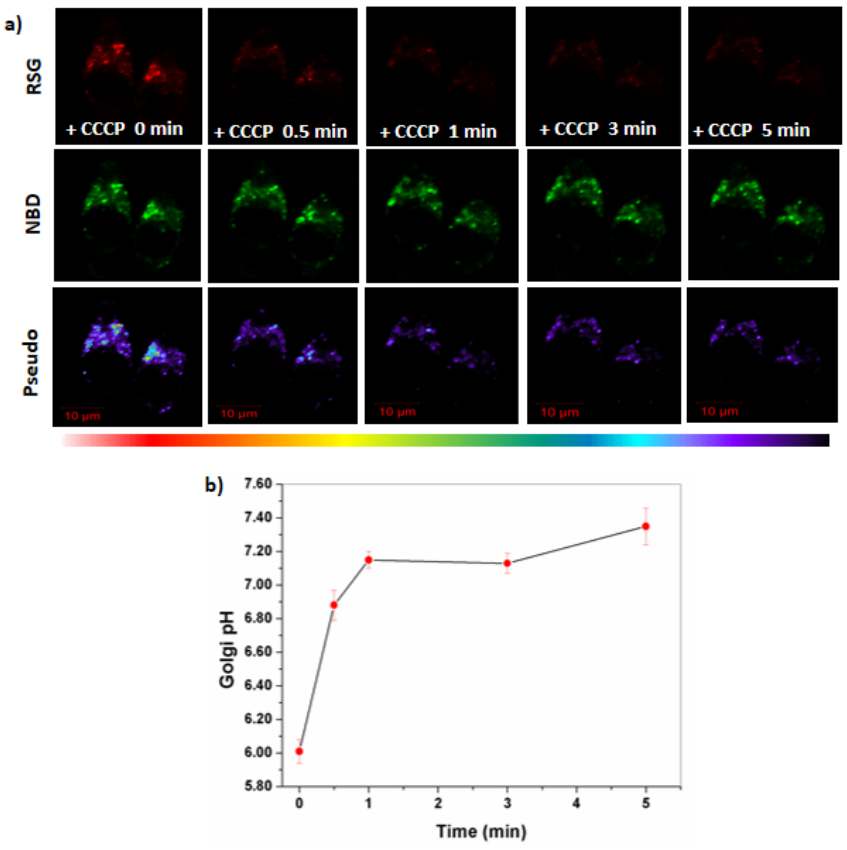


Fig. S13 (a) Real-time fluorescence images of SMMC-7721 cells co-incubated with **RSG** (10 μM) and NBD (5 μM) induced by CCCP (2 μM). (b) Effect of CCCP perfusion on Golgi pH at different time. Error bars represent mean deviation (± S.D.), n = 3. The red emission was collected from 568 to 650 nm for **RSG** (λ_{ex} = 561 nm). The green emission was collected from 493 to 550 nm for NBD (λ_{ex} = 488 nm). The pseudo-colour ratio images by mediating the red channel image with the green channel. The colour strip is the ratio bar. Scale bar: 10 μm.

Note: Carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 2 μM), a typical exogenous conductive H^+ ionophore that increasing the rate of dissipation of the pH_G gradient, caused a dramatic pH increase to 7.35 ± 0.11 after 5 min.

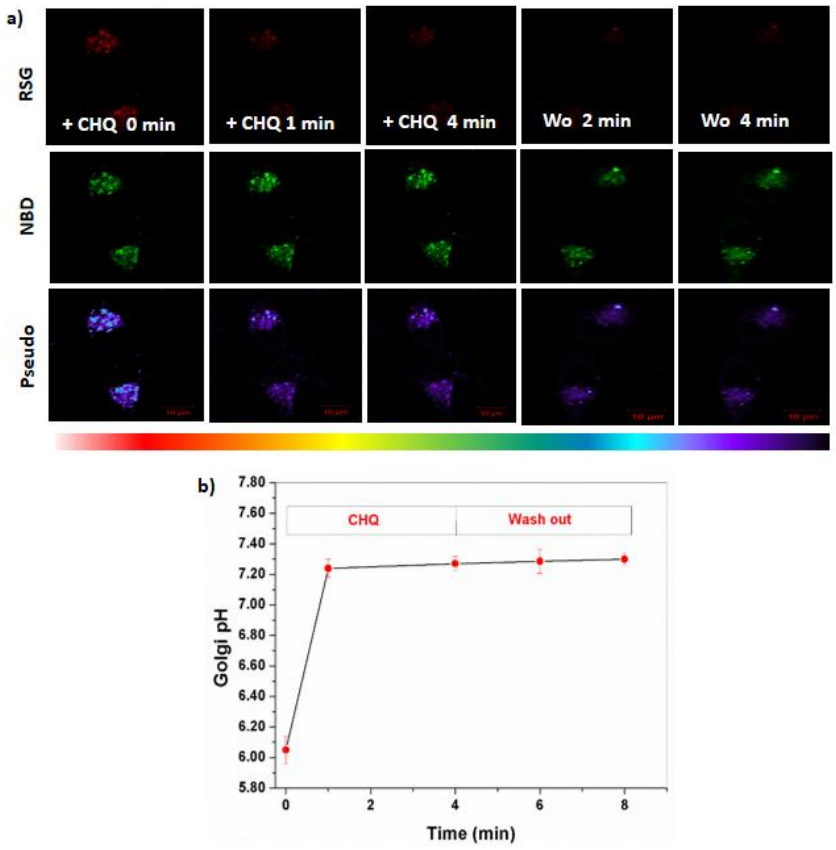


Fig. S14 (a) Real-time fluorescence images of SMMC-7721 cells co-incubated with **RSG** (10 μM) and NBD (5 μM) induced by chloroquine (100 μM). (b) Effect of chloroquine perfusion on Golgi pH at different time. Error bars represent mean deviation (± S.D.), n = 3. The red emission was collected from 568 to 650 nm for **RSG** (λ_{ex} = 561 nm). The green emission was collected from 493 to 550 nm for NBD (λ_{ex} = 488 nm). The pseudo-colour ratio images by mediating the red channel image with the green channel. The colour strip is the ratio bar. Scale bar: 10 μm.

Note: The antimalarial drug chloroquine (CHQ), a typical lysosomal toxin that leading to the alkalization of the lysosome, could also immediately alkalize pH_G to 7.24 ± 0.06 within only 1 min. However, washing out chloroquine had almost no effect on pH_G. This was consistent with the report from Thorens et al. (*Nature*, 1986, 321, 618) that the Golgi ulreastructural alterations could maintain 3 h after washing out the chloroquine, which were probably swollen lysosomes or endosomes, had disappeared.

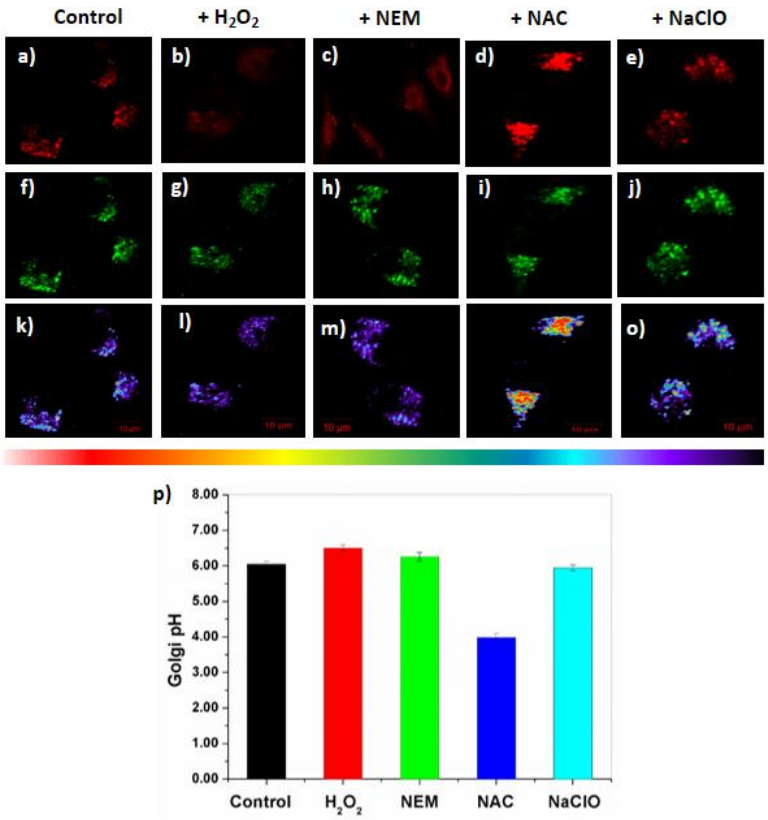


Fig. S15 Real-time fluorescence images of SMMC-7721 cells co-incubated with **RSG** (10 μM) and NBD (5 μM) induced by redox substances: control cells (a, f), and cells treated with 100 μM H_2O_2 (b, g), 100 μM NEM (c, h), 100 μM NAC (d, i) or 100 μM NaClO (e, j) for 20 min, respectively. (k) Golgi pH values of **RSG**-loaded SMMC-7721 cells treated with the above redox substances. Error bars represent mean deviation (± S.D.), n = 3. The red emission was collected from 568 to 650 nm for **RSG** (λ_{ex} = 561 nm). The green emission was collected from 493 to 550 nm for NBD (λ_{ex} = 488 nm). The pseudo-colour ratio images by mediating the red channel image with the green channel. The colour strip is the ratio bar. Scale bar: 10 μm.

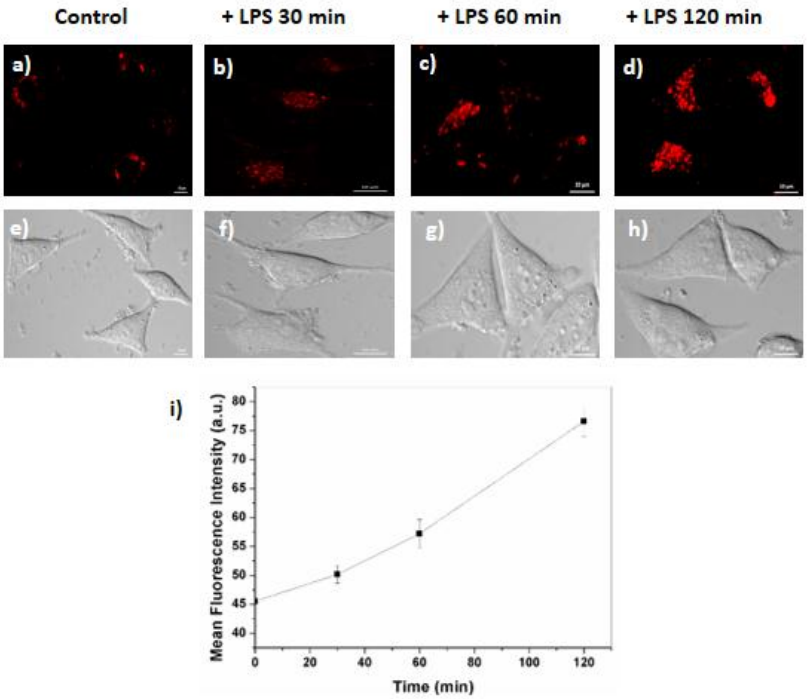
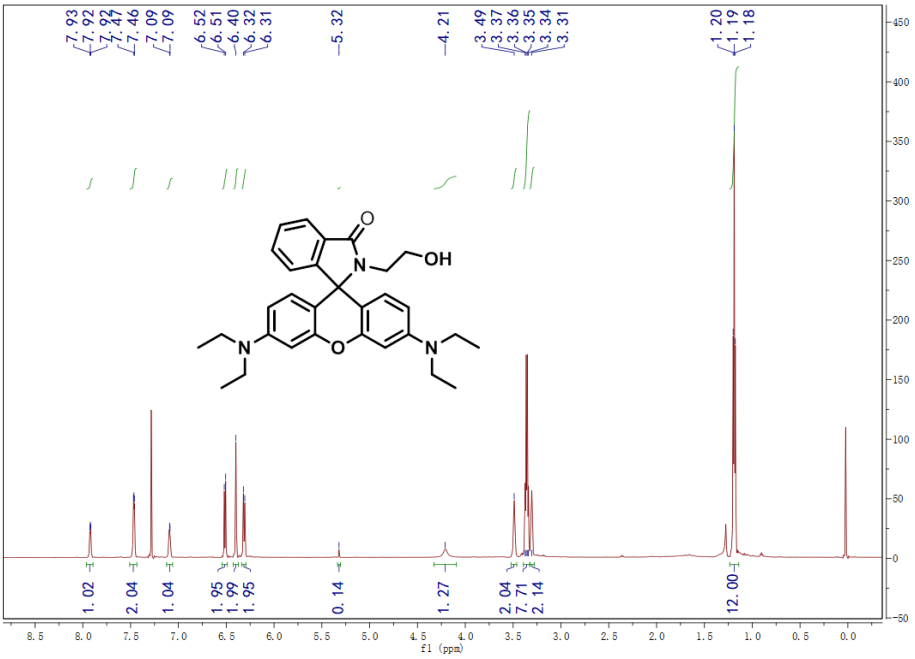
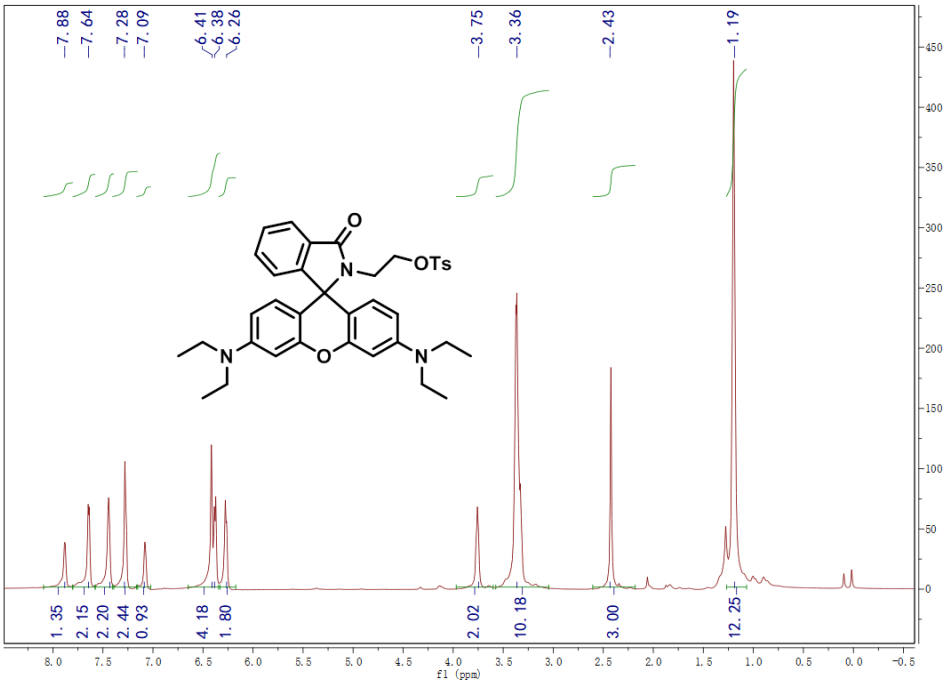
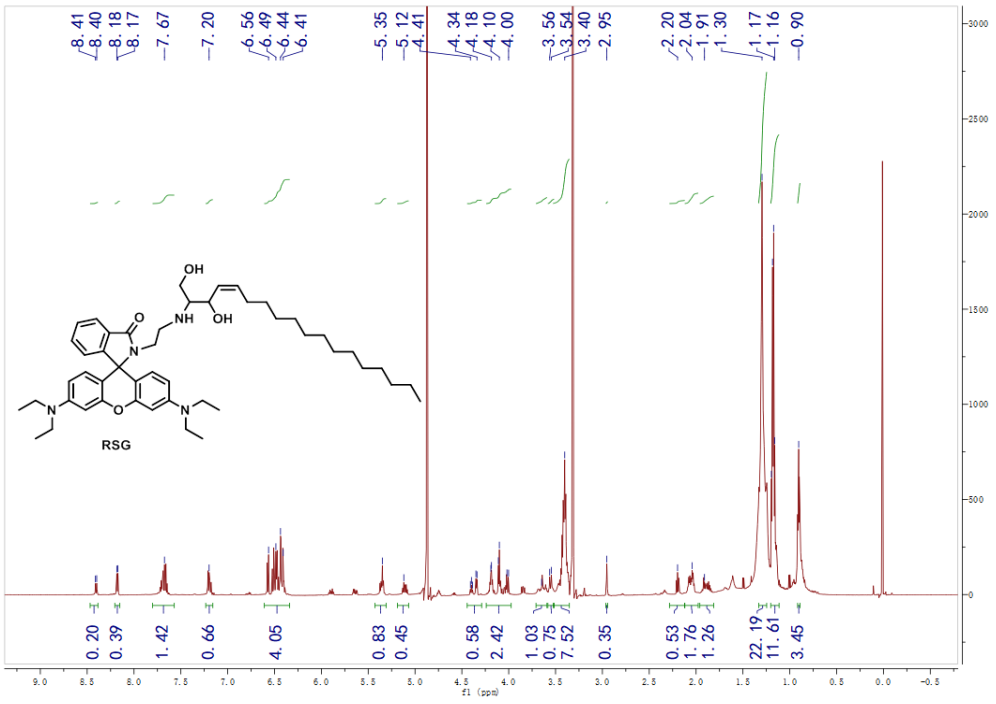


Fig. S16 Fluorescence images of **RSG** induced by LPS in live SMMC-7721 cells. (a) SMMC-7721 cells were incubated with only **RSG** (10 μM) for 10 min. (b-d) SMMC-7721 cells were mediated by LPS (50 μg/mL) for 30 min (b), 60 min (c), or 120 min (d), subsequently incubated with **RSG** (10 μM) for 10 min. (e-h) The bright-field cells images. (i) Mean fluorescence intensities of **RSG**-stained live cells. The red channel images were collected from 568 to 650 nm (λ_{ex} = 561 nm).

^{1}H NMR, ^{13}C NMR and HR-MS spectra analysis reports of Compound 1, Compound 2 and RSG



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8.17
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7.20
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6.41
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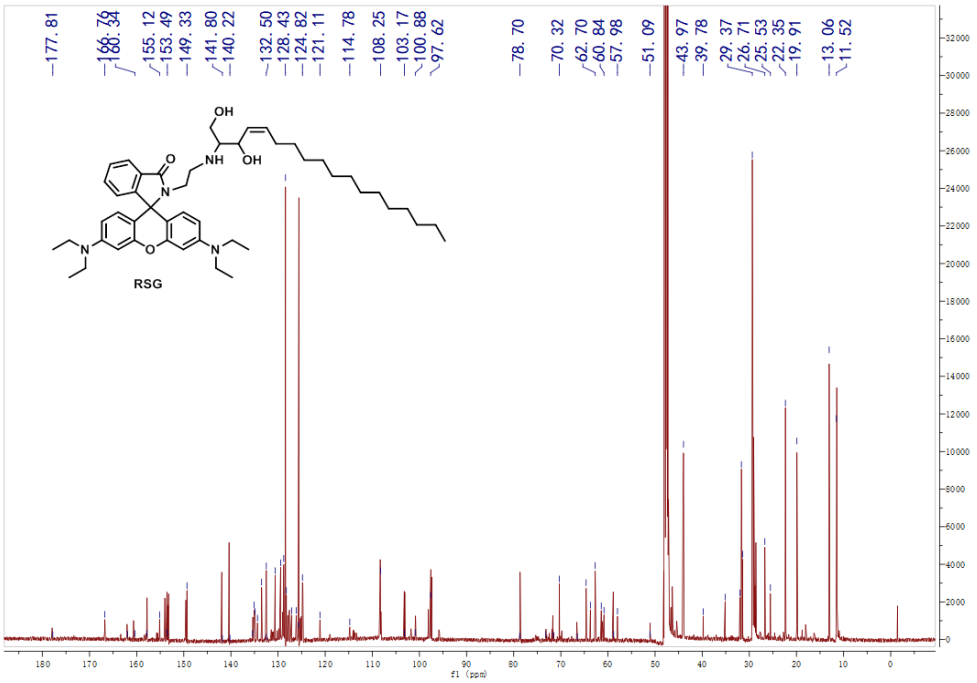
4.34
4.18
4.10
4.00
3.56
3.54
3.40
2.95
2.20
2.04
1.91
1.30
1.17
1.16
0.90
OH
NH
OH
O
N
O
N
N
RSG
0.20
0.39
1.42
0.66
4.05
0.83
0.45
0.58
2.42
1.03
0.75
7.52
0.35
0.53
1.76
1.26
22.19
11.61
3.45
f1 (ppm)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153.49
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43.97
39.78
29.37
26.71
25.53
22.35
19.91
13.06
11.52
OH
NH
OH
O
N
O
N
N
RSG
f1 (ppm)

HIGH RESLUTION MASS SPECTROMETRY REPORT

Sample	Formula (M)	Ion Formula	Measured m/z	Calc m/z	Diff (ppm)
RSG	$C_{48}H_{70}N_4O_4$	$[M+H]^+$	767.5460	767.5470	-1.30