Supplementary Materials for

Photochemically Controlled Activation of STING by CAIX-Targeting Photocaged Agonists to Suppress Tumor Cell Growth

Chunyong Ding,[1,7†*] Mengyan Du,[2,5,6†] Zhi Xiong,[1,4†] Xue Wang,[1†] Hongji Li,[1] Ende He,[1] Han Li,[2,6] Yijing Dang,[3] Qing Lu,[1] Shicong Li,[1] Ruoxuan Xiao,[1] Zhiai Xu,[3] Lili Jing,[1*] Liufu Deng,[1] Xiyuan Wang,[2] Meiyu Geng,[2,5] Zuoquan Xie,[2,5*] and Ao Zhang[1*]

[1] Shanghai Frontiers Science Center of Drug Target Identification and Delivery, College of Pharmaceutical Sciences, National Key Laboratory of Innovative Immunotherapy, Shanghai Jiao Tong University, Shanghai 200240, China.

[2] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai 201203, China.

[3] School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, China.

[4] School of Pharmacy, Nanchang University, Jiangxi 330000, China.

[5] University of Chinese Academy of Sciences, Beijing, 100049, China.

[6] School of Life Science and Technology, ShanghaiTech University, Shanghai, 200031, China.

[7] Zhangjiang Institute of Advanced Study, Shanghai Jiao Tong University, Shanghai 200240, China.

[†]These authors contributed equally to this work.

[*]Corresponding authors: chunding@sjtu.edu.cn; lilijing@sjtu.edu.cn; zqxie@simm.ac.cn; ao6919zhang@sjtu.edu.cn.

Chemical reagents and methods

All commercially available starting materials and solvents are reagent grade and used without further purification. Column chromatography was performed by using 300−400 mesh or 200−300 mesh silica gel. Analytical TLC was carried out employing silica gel 60 F254 plates, and spots were visualized by UV (254 or 365 nm). ^{1}H and ^{13}C NMR spectra were recorded with a Varian Mercury 300 or 400 MHz NMR spectrometer. Chemical shifts (δ) were reported in ppm downfield from an internal TMS standard, and J values were given in Hz. Low- and high-resolution mass spectra were obtained in the ESI mode from an Elite mass spectrometer. Purity of final compounds was determined by analytical HPLC, which was carried out on an Agilent Technologies 1260 series LC system with ultraviolet wavelengths in UV 254. HPLC analysis conditions are as follows: XDB-C18, 3.5 μm, 4.6 mm × 150 mm, and H_2O/MeOH or H_2O/MeCN and 0.1% TFA. All the assayed compounds showed a chemical purity of 95−100%. The light irradiation source in biological experiments is a model PR-CPC2-450 nm light purchased from Purui material technology Corporation Ltd., Shenzhen, China. The power of the 450 nm blue light is 6.7 mW/cm^2, and the distance of the light from the sample is 2 cm. Compounds MSA-2, **4** and **7** were prepared according to the literature procedures.[1-4]

Synthesis of *tert*-butyl (*E*)-3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2*H*-chromen-3-yl)acrylate (6)

To a solution of **4** (80 mg, 0.164 mmol, 1 eq) in THF and dichloromethane (4 mL, 1:1) was added tetrabutylammonium fluoride (1M in THF) (0.246 mL, 0.246 mmol, 1.5 eq). The reaction was stirred for 1 h, and then quenched with sat. NH_4Cl and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product, which was purified by column chromatography (silica gel, dichloromethane: ethyl acetate = 6:1) to give **6** in 70% yield (43 mg, 0.115 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 15.7 Hz, 1H), 7.68 (d, J = 9.1 Hz, 1H), 7.08 (d, J = 15.6 Hz, 1H), 6.63 (d, J = 9.3 Hz, 1H), 6.47 (s, 1H), 3.43 (q, J = 7.3 Hz, 4H), 1.51 (s, 9H), 1.22 (t, J = 7.1 Hz, 6H). LRMS (ESI): 374.1 [M+H$^+$].

Synthesis of (*E*)-3-(7-(diethylamino)-4-(((4-(5,6-dimethoxybenzo[*b*]thiophen-2-yl)-4-oxobutanoyl)oxy)methyl)-2-oxo-2*H*-chromen-3-yl)acrylic acid (1)

A solution of **6** (104 mg, 0.279 mmol, 1 eq) in DMF (3 mL) was treated with MSA-2 (98 mg, 0.334 mmol, 1.2 eq) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (80 mg, 0.419 mmol, 1.5 eq), 1-hydroxybenzotriazole and *N*,*N*-diisopropylethylamine (72 mg, 0.558 mmol, 2 eq). The reaction was stirred for 24 h, and then poured into water and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product, which was then purified by column chromatography (silica gel, petroleum ether: ethyl acetate = 2:1) to give (*E*)-(3-(3-(*tert*-butoxy)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl 4-(5,6-dimethoxybenzo[*b*]thiophen-2-yl)-4-oxobutanoate in 69% yield (125 mg, 0.192 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.75 (d, *J* = 15.6 Hz, 1H), 7.52 (d, *J* = 9.2 Hz, 1H), 7.23 (m, 2H), 7.07 (d, *J* = 15.5 Hz, 1H), 6.60 (dd, *J* = 9.2, 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 5.40 (s, 2H), 3.98 (s ,3H), 3.96 (s ,3H), 3.41 (q, *J* = 7.1 Hz, 4H), 3.33 (t, *J* = 6.7 Hz, 2H), 2.83 (t, *J* = 6.7 Hz, 2H), 1.51 (m, 9H), 1.21 (t, *J* = 7.1 Hz, 6H). LRMS (ESI): 672.2 [M+Na^+].

(*E*)-(3-(3-(*Tert*-butoxy)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl-4-(5,6-dimethoxybenzo[*b*]thiophen-2-yl)-4-oxobutanoate (146 mg, 0.224 mmol) was dissolved in dichloromethane (4 mL), and then trifluoroacetic acid (2 mL) was added and stirred for 1 h. After concentration under vacuum, the crude residue was purified by column chromatography (silica gel, dichloromethane: methanol = 25:1) to obtain **1** in 90% yield (120 mg, 0.202 mmol) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, *J* = 15.5 Hz, 1H), 7.84 (s, 1H), 7.56 (d, *J* = 9.3 Hz, 1H), 7.23 (m, 2H), 7.19 (d, *J* = 15.5 Hz, 1H), 6.62 (dd, *J* = 9.2, 2.6 Hz, 1H), 6.46 (d, *J* = 2.5 Hz, 1H), 5.42 (s, 2H), 3.97 (s, 3H), 3.95 (s, 3H), 3.43 (q, *J* = 7.1 Hz, 4H), 3.33 (t, *J* = 6.7 Hz, 2H), 2.83 (t, *J* = 6.7 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.83, 172.11, 170.59, 160.01, 156.13, 151.79, 151.31, 149.09, 147.68, 140.95, 137.61, 137.04, 132.77, 129.45, 127.28, 121.56, 114.40, 109.91, 108.08, 106.14,

103.69, 97.34, 77.41, 77.16, 76.91, 58.04, 56.35, 56.24, 45.09, 33.74, 28.54, 12.66. HRMS (ESI): calcd for $C_{31}H_{32}NO_9S$ [M+H$^+$] requires 594.1792, found 594.1776.

Synthesis of (*E*)-3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2*H*-chromen-3-yl)acrylic acid (5)

Compound **6** (84 mg, 0.224 mmol) was dissolved in dichloromethane (4 mL), and trifluoroacetic acid (2 mL) was added and stirred for 1 h. The crude material was then purified by column chromatography (silica, dichloromethane: methanol = 25:1) to obtain **5** in 90% yield (64 mg, 0.202 mmol) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.82 – 7.6 (m, 2H), 6.91 (d, *J* = 15.7 Hz, 1H), 6.78 (dd, *J* = 9.4, 2.6 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 5.62 (d, *J* = 4.7 Hz ,1H), 4.72 (d, *J* = 4.7 Hz, 2H), 3.47 (q, *J* = 7.1 Hz, 4H), 1.14 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.24, 159.74, 155.54, 153.75, 151.17, 136.47, 129.62, 128.15, 121.36, 111.57, 109.55, 107.48, 96.26, 55.20, 44.13, 12.37. HRMS (ESI): calcd for $C_{17}H_{20}NO_5$ [M+H$^+$] requires 318.1336, found 318.1342.

Synthesis of (*E*)-3-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylic acid (8)

Compound **4** (117 mg, 0.24 mmol) was dissolved in dichloromethane (4 mL) and trifluoroacetic acid (2 mL), and stirred for 1 h. After concentration under vacuum, the crude material was purified by column chromatography (silica gel, dichloromethane: methanol = 25:1) to obtain **8** in 79% yield (82 mg, 0.19 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 15.6 Hz, 1H), 7.66 (d, *J* = 9.3 Hz, 1H), 7.17 (d, *J* = 15.5 Hz, 1H), 6.63 (dd, *J* = 9.4, 2.5 Hz, 1H), 6.48 (d, *J* = 2.6 Hz, 1H), 4.93 (s, 2H), 3.44 (q, *J* = 7.0 Hz, 4H), 1.23 (t, *J* = 7.3 Hz, 6H), 0.91 (s, 9H), 0.16 (s, 6H).

Synthesis of (*E*)-*N*-(2-(2-(2-(3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2*H*-chromen-3-yl)acrylamido)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (9)

A solution of **8** (41 mg, 0.10 mmol) in DMF (2 mL) was treated with **7** (50 mg, 0.15 mmol, 1.5 eq) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (38 mg, 0.20 mmol, 2 eq), 1-hydroxybenzotriazole (27 mg, 0.20 mmol) and *N*,*N*-diisopropylethylamine (39 mg, 0.30 mmol). The reaction was stirred for 24 h, and then poured into water and extracted into ethyl acetate. The combined organics

were dried over $MgSO_4$, filtered and concentrated to obtain the crude product, which was purified by column chromatography (silica gel, petroleum ether: ethyl acetate = 1:1) to give (*E*)-*N*-(2-(2-(2-(3-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamido)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide in 78% yield (58 mg, 0.078 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 4H), 7.77 (d, *J* = 15.2 Hz, 1H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.44 (s, 1H), 7.18 (d, *J* = 15.2 Hz, 1H), 6.63 (dd, *J* = 9.3, 2.5 Hz, 1H), 6.60 (s, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 5.85 (s, 2H), 4.94 (s, 2H), 3.76 – 3.34 (m,16H), 1.22 (t, *J* = 7.1 Hz, 6H), 0.88 (s, 9H), 0.15 (s, 6H).

To a solution of (*E*)-*N*-(2-(2-(2-(3-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamido)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (80 mg, 0.108 mmol, 1 eq) in THF and dichloromethane (4 mL, 1:1) was added tetrabutylammonium fluoride (1M in THF) (0.161 mL, 0.162 mmol, 1.5 eq). The reaction was stirred for 1 h, and then quenched with sat. NH_4Cl aqueous solution and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product, which was purified by column chromatography (silica gel, dichloromethane: ethyl acetate = 6:1) to give **9** in 68% yield (46 mg, 0.073 mmol) as an orange solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (s, 4H), 7.84 – 7.78 (m, 2H), 7.25 (d, *J* = 15.3 Hz, 1H), 6.80 (dd, *J* = 9.3, 2.6 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 4.91 (s, 2H), 3.71 – 3.67 (m, 6H), 3.63 – 3.59 (q, *J* = 5.4 Hz, 4H), 3.54 – 3.46 (m, 6H), 1.23 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, CD_3OD) δ 169.39, 168.64, 162.47, 156.95, 153.83, 152.63, 147.39, 138.66, 133.22, 128.74, 128.57, 127.05, 125.07, 113.55, 110.66, 109.10, 97.42, 71.08, 71.06, 70.31, 70.20, 56.39, 45.44, 40.76, 40.31, 12.50. HRMS (ESI): calcd for $C_{30}H_{39}N_4O_9S$ [$M+H^+$] requires 631.2432, found 631.2425.

Synthesis of (*E*)-(7-(diethylamino)-3-(1,12-dioxo-1-(4-sulfamoylphenyl)-5,8-dioxa-2,11-diazatetradec-13-en-14-yl)-2-oxo-2*H*-chromen-4-yl)methyl 4-(5,6-dimethoxybenzo[*b*]thiophen-2-yl)-4-oxobutanoate (2)

A solution of **9** (176 mg, 0.279 mmol, 1 eq) in DMF (3 mL) was treated with MSA-2 (98 mg, 0.335 mmol, 1.2 eq) in the presence of 1-(3-dimethylaminopropyl)-3-

ethylcarbodiimide hydrochloride (80 mg, 0.419 mmol, 1.5 eq), 1-hydroxybenzotriazole and *N*,*N*-diisopropylethylamine (72 mg, 0.558 mmol, 2 eq). The reaction was stirred for 24 h, and then poured into water and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product, which was purified by column chromatography (silica gel, petroleum ether: ethyl acetate = 1:1) to give **2** in 69% yield (174 mg, 0.192 mmol) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.86 (m, 5H), 7.71 (d, *J* = 15.2 Hz, 1H), 7.55 (d, *J* = 9.3 Hz, 1H), 7.38 (s, 1H), 7.23 (m, 2H), 6.99 (d, *J* = 15.2 Hz, 1H), 6.68 – 6.58 (m, 2H), 6.48 (s, 1H), 5.85 (s, 2H), 5.38 (s, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 3.77 – 3.63 (m, 8H), 3.57 (t, *J* = 5.5 Hz, 2H), 3.40 (m, 6.7 Hz, 8H), 2.83 (t, *J* = 6.5 Hz, 2H), 1.22 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.37, 172.66, 166.68, 166.64, 161.01, 155.55, 151.33, 149.05, 146.70, 145.07, 140.78, 138.32, 137.01, 132.84, 132.15, 130.03, 127.94, 127.20, 126.68, 125.61, 115.17, 110.27, 106.26, 103.69, 70.35, 70.20, 69.56, 69.42, 58.59, 56.36, 56.24, 45.33, 39.77, 33.71, 28.48, 12.58. HRMS (ESI): calcd for $C_{44}H_{51}N_4O_{13}S_2$ [M+H$^+$] requires 907.2889, found 907.2910.

Photolysis assay for compounds 1 and 2

Compounds **1** or **2** was dissolved in CH_3CN/PBS (1/1, v/v) buffer solution, and then was irradiated by 450 nm blue light for photolysis in a cell phototoxicity irradiator (model: PR-CPC2-450 nm light) purchased from Purui material technology Corporation Ltd., Shenzhen, China. The power of the 450 nm blue light is 6.7 mW/cm^2, and the distance of the light from the sample is 2 cm. After irradiation for a while, the solution was analyzed by HPLC, which was carried out on an Agilent Technologies 1260 series LC system with ultraviolet wavelengths in UV 254 nm. HPLC analysis conditions are as follows: XDB-C18, 3.5 μm, 4.6 mm × 150 mm, and H_2O/MeOH and 0.1% TFA.

Biological reagents

QUANTI-Luc solution (cat. code: rep-qlc2) was purchased from InvivoGen (San Diego, USA). Antibodies against phospho-TBK1 (Ser172) (cat. code: 5483S), TBK1 (cat. code: 3504S), phospho-IRF3 (Ser396) (cat. code: 4947S), IRF3 (cat. code: 4302S), and GAPDH (cat. code: 5174S) were purchased from Cell Signaling Technology

(Beverly, MA). MACS Running Buffer (cat. code: 130-091-221) was purchased from Miltenyi. Human IFN-β ELISA Kit (cat. code: 70-EK1236-96) was purchased from Multisciences. Human IP-10 ELISA Kit (cat. code: 550926) was purchased from BD Bioscience.

Cell lines

THP-1-Dual cells (cat. code: thpd-nfis) and THP1-Dual-KO-STING cells (cat. code: thpd-kostg) were purchased from InvivoGen (San Diego, USA). CT26 colon cancer cells (cat. code: CRL-2638) and HT29 cancer cells (cat. code: HTB-38) were purchased from ATCC. THP-1-Dual cells and THP1-Dual-KO-STING cells were cultured in RPIM-1640 medium with 10%FBS, containing 100 μg/mL of Zeocin (cat. code: ant-zn-05, InvivoGen), 50 μg/mL of Normocin (cat. code: ant-nr-1, InvivoGen) and 10 μg/mL of blasticidin (cat. code: ant-bl-1, InvivoGen). CT26 cells were cultured in the Roswell Park Memorial Institute 1640 (RPMI-1640, Gibco) medium with 10% fetal bovine serum (FBS, Gibco). HT29 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% FBS. All cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2.

ISG-reporter assay

THP1-Dual cells or THP1-Dual-KO-STING cells (1×105 cells/well) were seeded in 96-well plates with 180 μL medium, and then treated with compounds at different concentrations (20 μL) for 1 h. The cells were irradiated with 450 nm blue light for indicated times, and then incubated for 24 h in the incubator. Subsequently, 20 μL of supernatant and 50 μL of QUANTI-Luc detection reagent were added to 96-well black plate (catalog number: 3601; Corning). The luminosity was measured by SpectraMAX Paradigm.

Western blot analysis

THP1-Dual cells (1×10^6 cells/well) were seeded into a 6-well plate, and treated with compounds (2.5, 5 and 10 μM) for 1 h. Then, the cells were irradiated with 450 nm blue light for 15 min and successively incubated for 4 h. The cell lysate was collected using 1x SDS-PAGE sample loading buffer (catalog number: P0015; Beyotime, China) and boiled at 100 °C for 30 min. Equal cell lysate was electrophoresed on 10% SDS-PAGE

gel and transferred to nitrocellulose membranes. The membrane was blocked for 1 h at room temperature with 5%BSA in 1xTris-buffered saline Tween-20 (TBST) (25 mM Tris, 150 mM NaCl, 2 mM KCl, pH 7.4, supplemented with 0.1% Tween-20). Then, the membrane was probed with anti-TBK1, anti-phospho-TBK1 (Ser172), anti-IRF3, anti-phospho-IRF3 (Ser396) and anti-GAPDH overnight at 4 °C. After washing the membranes with TBST, horseradish peroxidase-conjugated anti-rabbit IgG (1: 2000) (cat.log111-035-003, Jackson) or anti-mouse IgG (1: 5000) (cat.log115-035-003, Jackson) antibodies were incubated at room temperature for 1 h. The membranes were washed with TBST, and the bands were developed with an enhanced chemiluminescence assay (Thermo Scientific) or Femto chemiluminescence assay (Thermo Scientific) in LMAGE QUANT LAS4 (GE healthcare).

CT26 or HT29 cells were collected under hypoxia (0.5% O_2) using Ruskinn Invivo$_2$ 400 Hypoxia Workstation (Fannin Healthcare, Ireland) or normoxia for indicated times, and protein samples were extracted and subjected to western blotting analysis. The protocol was similar to the above method, and mouse CAIX antibody (cat. code: AF2344, R&D Systems) and human CAIX antibody (cat. code: NB100-417, Biotechne) were used for CT26 and HT29 cells, respectively.

ELISA assay

THP1-Dual cells (1×106 cells/well) were plated into a 6-well plate, and treated with compounds at indicated doses, and the cells were irradiated with 450 nm blue light for 15 min, and then incubated for 24 h. The cell supernatant was tested using human IFN-β and IP-10 detection kit according to the manufacture's protocol.

RNA interference

siRNAs targeting mouse CAIX were purchased from Genepharma (Shanghai, China). The siRNA sequence for CAIX is CCUGGAACUUCUGGGUUAUTT-AUAACCCAGAAGUUCCAGGTT. Transfection of siRNA was performed using Lipofectamine RNAiMAX Transfection Reagent (cat. code:13078075, Invitrogen, USA) according to the manufacturer's instructions. Briefly, CAIX siRNA or control siRNA were transfected into 8×10^4 CT-26 cells. After 6 h, the cell culture medium was replaced with the fresh one. After another 72 h, the cells were collected and subjected

to flow cytometry assay. Meanwhile, the efficiency of CAIX knock down was verified by western blot analysis. The protocol was similar to the above method, except the primary antibodies including anti-mouse CAIX (R&D Systems, cat. code:AF2344, dilution, 1: 1000) and anti-GAPDH.

Flow cytometry assay

CT26 or HT29 cells (2.5×10^5 cells/well) were harvested and washed twice with pre-cooled PBS after trypsinization. The cells were incubated with compounds (10 μM) or vehicle (0.1% DMSO) on ice for 40 min, and then the cells were washed with MACS buffer for three times, and the fluorescence was detected under the 488 channel and analyzed on a FACS Calibur flow cytometer (BD Bioscience). For CAIX siRNA assay, the experiment step was shown as above, and then cells were incubated with compound **2** (10 μM) or vehicle (0.1% DMSO) on ice for 40 min and washed with MACS buffer and the fluorescence was detected by FACS Calibur flow cytometer (BD Bioscience). For acetazolamide competitive assay in CT26 cells, the cell pellets were added with or without 10 μM of acetazolamide and incubated on ice for 30 min. After washing twice with MASC buffer, the cells were incubated with compound **2** or vehicle on ice for 40 min, and then washed with MACS buffer for three times, and the fluorescence was detected. For acetazolamide competitive assay in HT29 cells, the protocol was like that of CT26 cells, except for 100 μM of acetazolamide was used and without wash step before the addition of compound **2,** and the fluorescence was detected by CytoFLEX S (Beckman Coulter. Inc).

For hypoxia assay, CT26 or HT29 cells (2.5×10^5 cells/well) were incubated under hypoxia (0.5% O_2) for 24 h, and under normoxia as control. Next, Cells were harvested and washed twice with pre-cooled PBS after trypsinization and incubated with vehicle (0.1% DMSO) or **2** (10 μM) on ice for 10 min, and then washed with MACS buffer and the fluorescence was detected by CytoFLEX S (Beckman Coulter. Inc). FlowJo software was used for data analysis.

Zebrafish maintenance and embryo handling

The wild type (WT) AB and transgenic zebrafish were maintained, handled, and bred according to the standard protocols from the Institutional Animal Care Committee of

Shanghai Jiao Tong University. Adult zebrafish were raised in a circulating water system under a 14h/10h light/dark cycle at 26-28 °C. Adult male and female zebrafish were mated in the morning and the embryos were collected and kept at 28.5 °C in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgSO_4$). Embryos were staged by days post-fertilization (dpf) and days post-tumor cell injection (dpi). Tg (*coro1a*:EGFP) zebrafish was kindly provided by Professor Li Li from Southwest University, China. AB strain and Tg(*mpeg1*:mCherry) zebrafish were obtained from China zebrafish resource center.

Embryo lethality and toxic studies

WT embryos at 1 dpf were dechorionated by pronase (Roche), and treated with DMSO, MSA-2 (40 μM) or **2** (10 μM) until 5 dpf. E3 medium and chemical solutions were changed every 24 h. The embryos were examined under a stereomicroscope every 24 h. For each chemical treatment, half of the group were exposed to light intensities of 6.7 mW/cm^2 by a cell phototoxicity irradiator (PR-CPC2-450 nm, Purui material technology Corporation Ltd., Shenzhen, China) for 30 min at 4 dpf and returned to the incubator. At 5 dpf, all embryos were anesthetized with 0.02% tricaine (Sigma), and imaged under SZX16 stereomicroscope (Olympus, USA). The embryos were then used by real-time qPCR analysis to analyze the expression level of apoptotic genes. See "Gene expression analysis by real-time qPCR" for detailed methods and the primers used for different genes.

FACS cell sorting

Tg (*coro1a*:EGFP) embryos at 5 dpf with and without treatment were washed in the sterile PBS solution, shredded with a blade and incubated for 20 min (37 °C) with 38 μg/mL Liberase (Roche, Germany). 10% FBS was added to stop the reaction, followed by filtration (40 μm filter) and centrifugation (1500 rpm, 4 °C, 10 min). The supernatant was removed, and the single cells were resuspended with 800 μL PBS with 2% FBS. GFP negative and positive cells were sorted into PBS solution with FACS Aria II (Becton, Dickinson and Company, USA).

Gene expression analysis by real-time qPCR

For gene expression analysis, 5-dpf zebrafish whole embryos or sorted corola:GFP$^+$ cells were lysed in TRIzol reagent (Thermo Fisher Scientific, USA), and total RNAs were extracted according to the manufacture's protocol. For gene expression analysis in Figure 5C, Tg (*coro1a*:EGFP) embryos at 4 dpf were treated with DMSO (1%), MSA-2 (40 μM) or **2** (10 μM) for 24 hours. For **2**-treated embryos, half of the group were exposed to 450 nm light intensities of 6.7 mW/cm^2 for 30 min. All embryos were incubated at 28.5 °C for another 3 h, and then anesthetized. The corola:GFP$^+$ cells from whole embryos for each group were FACS sorted and collected and lysed in TRIzol reagent and total RNAs were extracted.

cDNAs were synthesized from total RNA using the Hifair III 1st Strand cDNA Synthesis SuperMix reagent Kit (Yeasen, Shanghai, China). Hieff qPCR SYBR Green Master Mix (Yeasen, Shanghai, China) was used for qPCR analysis according to manufacturer's protocol. Each target gene was calculated using the 2−ΔΔCT method as in the reference[5]. The relative expression of STING in different cell populations in Fig 5A was normalized to *β*-actin. The primers for different target genes and *β*-actin (reference gene) are listed in Table S1.

Table S1. Real-time qPCR primer sequence

Primer	Nucleotide sequence 5'- 3'
STING F	TTCTCTGGAGTGTCGGAACC
STING R	GATCACCTGTGTGCTCATCG
NF-κB F	CATTCCCTACGGCTAAACGA
NF-κB R	AGAAAAAGGAGGTGGGTGGA
VIPERIN F	GCTGAAAGAAGCAGGAATGG
VIPERIN R	AAACACTGGAAGACCTTCCAA
TNF-α F	GCGCTTTTCTGAATCCTACG
TNF-α R	TGCCCAGTCTGTCTCCTTCT
IL-6 F	TGGACTTCGCAGCAC AAAATG
IL-6 R	GTTCACTTCACGCTCTTGGATG
Tp53α F	ACCACTGGGACCAAACGTAG
Tp53α R	CAGAGTCGCTTCTTCCTTCG
Bax F	GGCTATTTCAACCAGGGTTCC
Bax R	TGCGAATCACCAATGCTGT
Caspase3 F	ATGCCAAGCCTCAATCCC
Caspase3 R	TCACAATGTATCCAAGCTTTCG
Mdm2 F	AAGCAGTGATCCTGAGAGTTC
Mdm2 R	ATCCGAAGACTCGCTGTTC

β-actin F	AGAGCTATGAGCTGCCTGACG
β-actin R	CCGCAAGATTCCATACCCA

Confocal imaging analysis of macrophage proliferation

4 dpf Tg(*mpeg1*: mCherry) embryos were exposed to DMSO, MSA-2 (40 μM) or **2** (10 μM) for 12 h. For each treatment, half of the group were exposed to light intensities of 6.7 mW/cm^2 for 30 min and returned to incubator for additional 12 h. At 5 dpf, all embryos were anesthetized, and mounted in 1% low-melt agarose (Shuhong Biotechnological Corporation Ltd., Shanghai, China) and imaged under a confocal microscope Leica SP8 microsystems (Leica, Germany). Mean value of mCherry fluorescence intensities for each group were analyzed using ImageJ software.

Cell staining and zebrafish tumor cell xenografts

CT26 and HT29 cells were labeled with CM-DiI (Invitrogen) for 15 min at 37 ℃ and then 20 min at 4 ℃. The labeled cells were washed in 100% FBS and twice in 67% DPBS. The cells were then resuspended in 4% PVP-K30 (Polyvinylpyrrolidone K30, solarbio, China) and the final cell concentration was 1×10^7 cells/mL with the 97% cell viability. Zebrafish embryos at 2 dpf were anesthetized with 0.02% tricaine (sigma) and mounted on the left side of low-melting-point agarose pad. Then, CT26 and HT29 cells in a volume of 5 nL were injected into the yolk of 2-dpf embryos under SZX16 stereomicroscope (Olympus, Tokyo, Japan). After that, the injected zebrafish were kept at 32 ℃ for 2 h. The successful zebrafish xenografts were selected under the fluorescence microscope and maintained at 34 ℃ to the end. The embryos were examined under a MVX10 Macro Zoom Fluorescence Microscope System (Olympus, Japan) every 24 h.

Drug treatment for zebrafish tumor cell xenografts

CT26 or HT29 cells were injected in 2-dpf zebrafish. The successful zebrafish xenografts were taken images at 3 dpf (i.e. 1 day post injection, dpi) and then treated with drugs (DMSO, MSA-2, 40 μM or **2,** 10 μM) for consecutive 3 days until 4 dpi. The chemical solutions were changed every 24 h. At 3 dpi, the half of group with each chemical treatment were exposed to light (6.7 mW/cm^2 at 450 nm) from a cell

phototoxicity irradiator (PR-CPC2-450 nm, Purui material technology Corporation Ltd., Shenzhen, China) for 30 min and returned to the incubator. At 4 dpi, all embryos were anesthetized with 0.02% tricaine (Sigma), and imaged under a confocal microscope Leica SP8 microsystems (Leica, Germany).

Imaging analysis of zebrafish tumor cell xenografts

Animals in the same experiments were imaged under the same conditions on a confocal microscope Leica SP8 microsystems (Leica, Heidelberg, Germany). A 5-μm interval in a total of ~100 μm stack images were acquired using the Z-stack function[6]. All images were acquired in the same focal plane in bright field and the transmitted light passing through the mCherry filter (566-650 nm) for Dil and the GFP (493-540nm) filter. Each z-stacks were projected using maximum intensity projection to obtain 2D images. The tumor size was quantified by measuring the area and fluorescence intensity of 2D image with ImageJ software according to previous studies[7-9]. The tumor size was evaluated at 1 dpi and 4 dpi, and the relative tumor growth was calculated as the ratio of the tumor size at 4 dpi to 1 dpi. In each group, the relative tumor growth in xenografted embryos after different drug treatments was normalized to the control DMSO-treated xenografted embryos, similar to previous reported methods[10-11]. To calculate the tumor cells co-located with coro1a:GFP^+ cells, the number of green coro1a:GFP^+ cells, or Dil labelled tumor cells in the images were qualified using ImageJ software Cell counter plugin.

Statistics method

GraphPad Prism 8.0.2 (GraphPad Software, Inc.: San Diego, CA, USA, 2019, https://www.graphpad.com) was used to analyze all data. The values of all experiments are presented as mean ± SEM. The statistical significance was analyzed using unpaired Student's t-test. The statistical significance was displayed as "ns" for no statistical significance, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$ were considered statistically significant as indicated.

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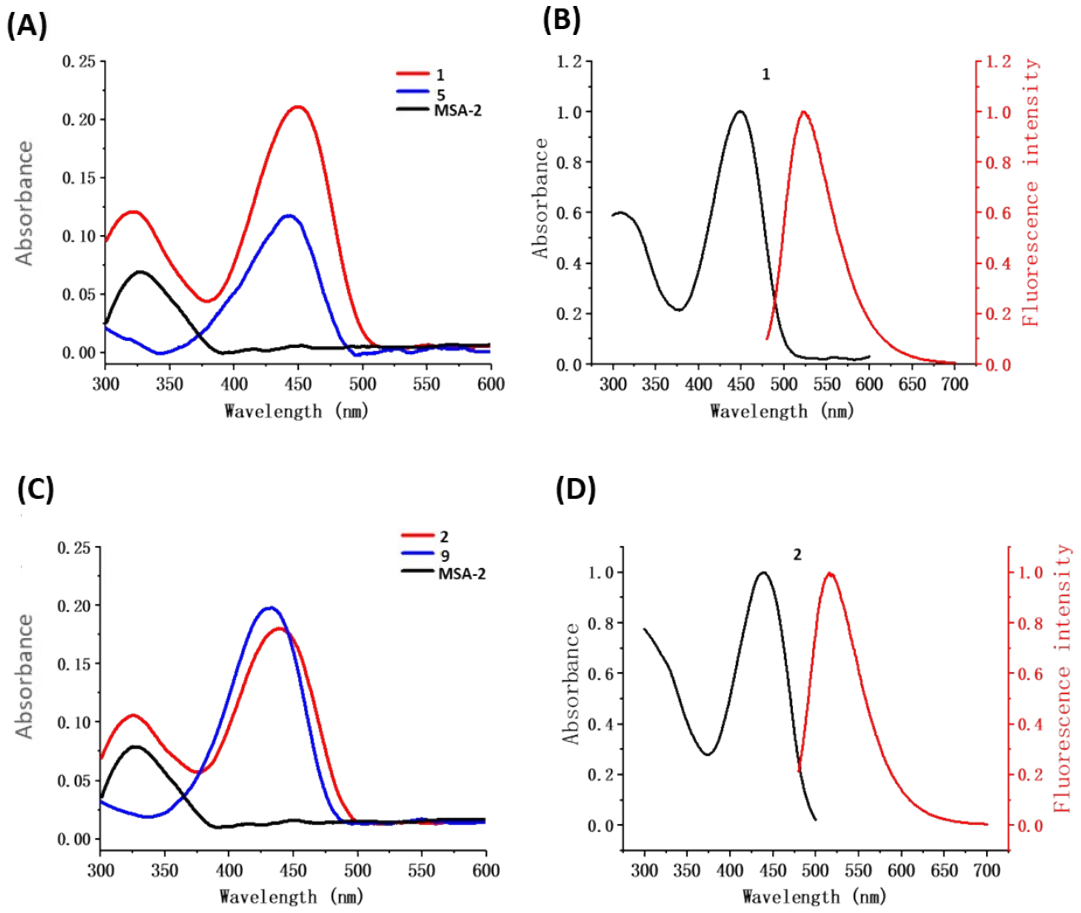

Fig. S1. (A) UV−vis absorption spectra of **1**, PPG (**5**), and **MSA-2** in buffer; (B) Absorption (left, black) and fluorescence (right, red) spectra of **1** in buffer (λex = 450 nm); (C) UV−vis absorption spectra of **2**, PPG (**9**), and **MSA-2** in buffer; (D) Absorption (left, black) and fluorescence (right, red) spectra of **2** in buffer (λex = 450 nm).

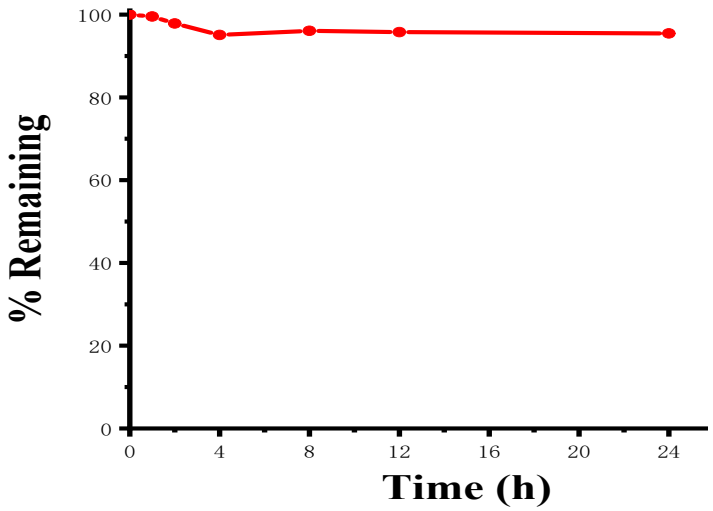

Fig. S2. Chemical stability of **2** in a mixture of 5% DMSO in PBS (pH 7.4) at a concentration of 50 μM at 37 °C. Experiments were performed in triplicates (n = 3) and analyzed by HPLC (λ = 450 nm).

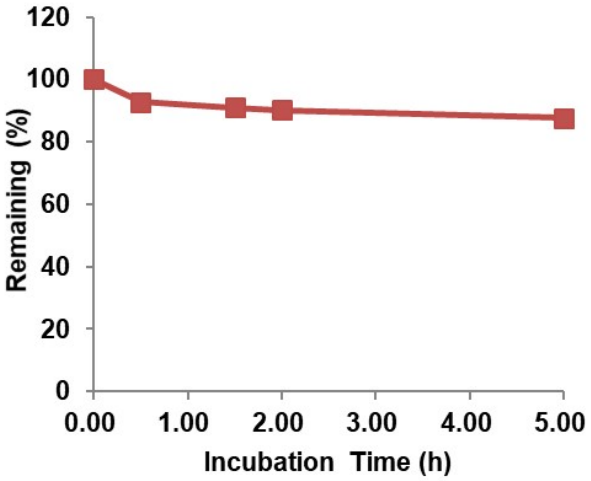

Fig. S3. The stability of **2** in human plasma. Experiments were performed in 96-well plates with final incubation volume of 320 µL per well. Each well contained 156 µL plasma, 8 µL test compound 40 µM and 156 µL PBS (pH 7.4). Plates were incubated at 37°C, and aliquots (50 µL) of incubation medium were collected at indicated times and dispensed in 96-well plates containing 200 µL of acetonitrile. After reactions were terminated, plates were centrifuged and supernatants were analyzed by LC-MS/MS. Experiments were run in duplicates (n = 2).

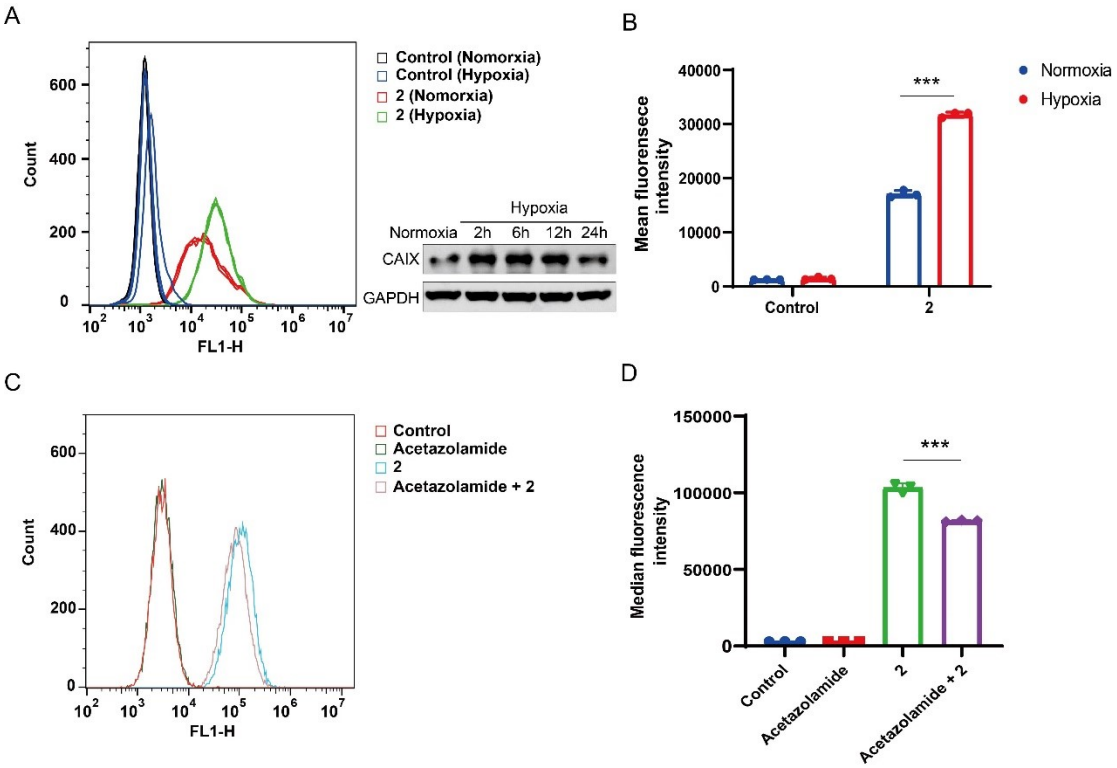

Fig. S4. (A) HT29 cells were incubated under normoxia and hypoxia (0.5% O_2), and 24 h later the cells were incubated with or without **2** (10 μM) and subjected to flow cytometry analysis, besides the expression of CAIX was determined by western blotting at different time points; (H) Median fluorescence intensity of (B). (C) Flow cytometric analysis of HT29 cells treated with acetazolamide (100 μM) and **2** (10 μM), as well as the acetazolamide-preincubated HT29 cells treated with **2**; (D) Median fluorescence intensity of (C). Results are expressed as mean ± SEM from two independent experiments (***$p < 0.01$, t-test).

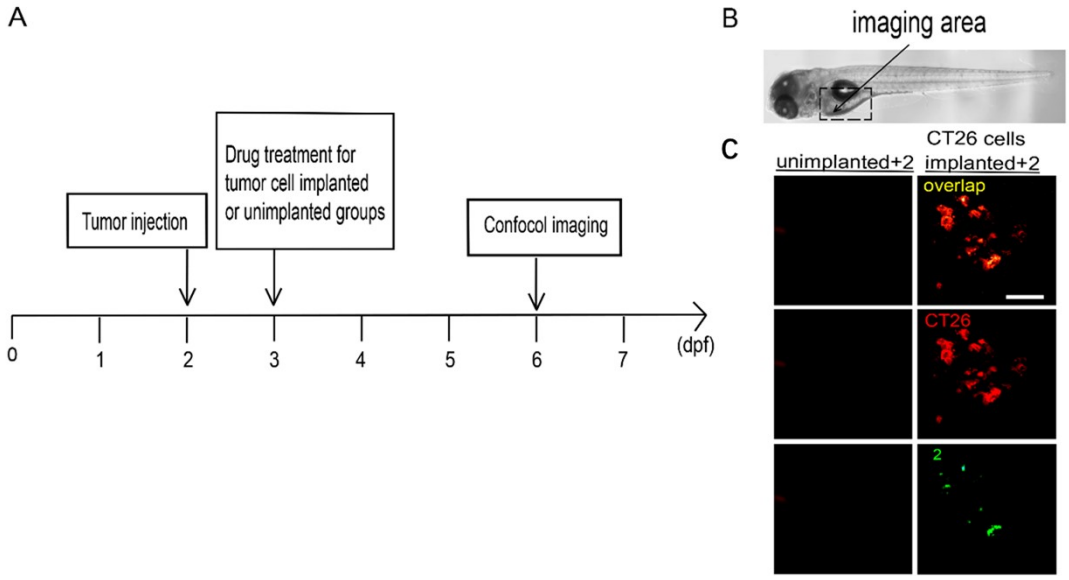

Fig. S5. Compound **2** targets CT26 cells in zebrafish xenografts. (A) The time scheme for the experiments in (B-C). Compound **2** (10 μM) was added to the embryo medium. (B) Scheme of the tumor cell injection site and subsequent imaging area. (C) Representative confocal images of CT26 tumor cells and **2** in zebrafish xenografts at 4 dpi. Scale bar in C: 25 μm; dpf, days post fertilization.

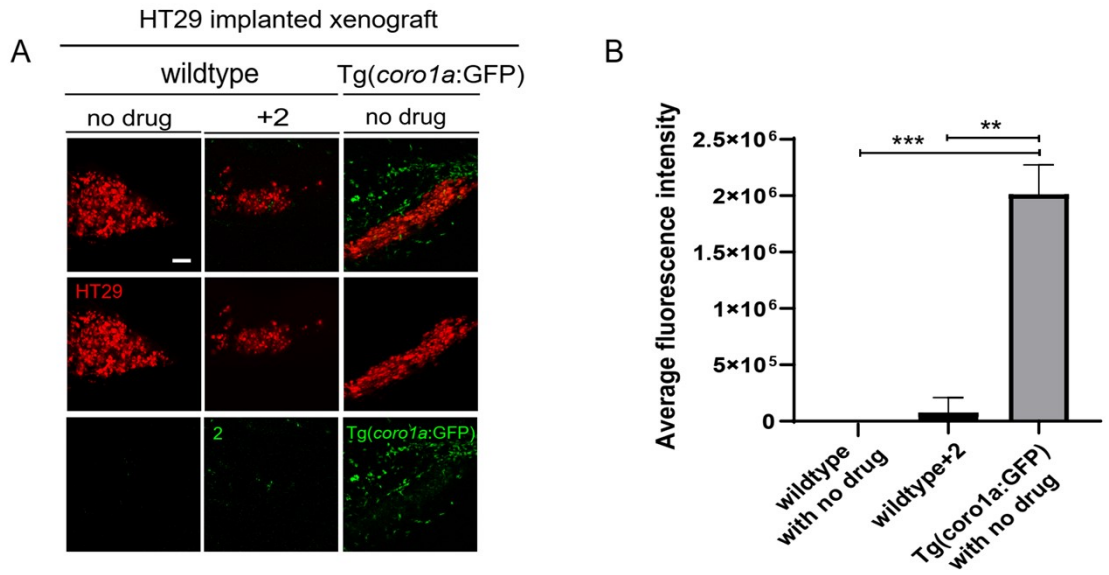

Fig. S6. Comparison of fluorescence intensity between **2** and Tg(*coro1a*:GFP) in zebrafish. (A) Representative confocal images of HT29 cells and **2** (10 μM) in zebrafish WT xenografted embryos, and of HT29 cells and corola:GFP in Tg(*coro1a*:GFP) xenografted embryos at 4 dpi. (B) Summary of fluorescent intensity of **2** and corola:GFP from (B), obtained by the excitation wavelength of 488 nm (λex = 488 nm). Results are expressed as mean ± SEM from multiple experiments, n = 3-4 embryos per condition, (***$p < 0.001$, **$p < 0.01$, t-test). Scale bar in A: 50 μm; dpi, days post injection.

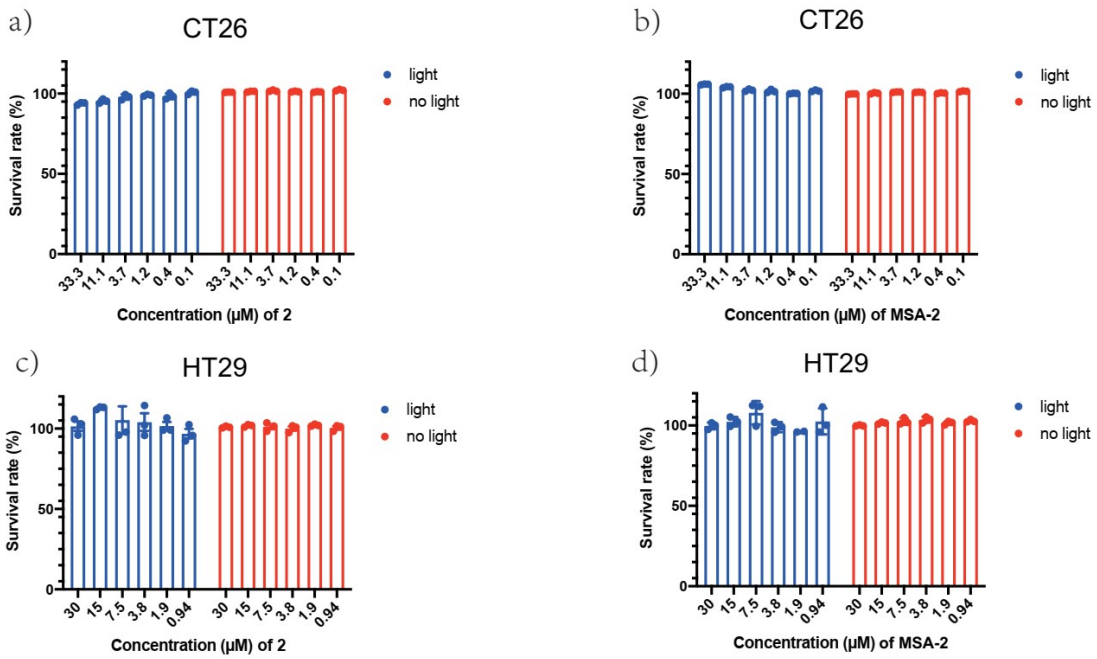

Fig. S7. Cytotoxic effects of **2** and MSA-2 on cancer cell lines. (a, b) CT26 cancer cells were treated with various concentrations of compound **2** or MSA-2 for 72 h with or without 15 min light exposure, and the cell viability was determined by sulforhodamine B (SRB) method, the survival rate (%) was calculated relative to the vehicle control; (c, d) HT29 cells were treated with various concentration of compound **2** or MSA-2 for 24 h with or without 15 min light exposure, and the cell viability was determined by SRB, the survival rate (%) was calculated relative to the vehicle control. Experiments were run in triplicates (n = 3).

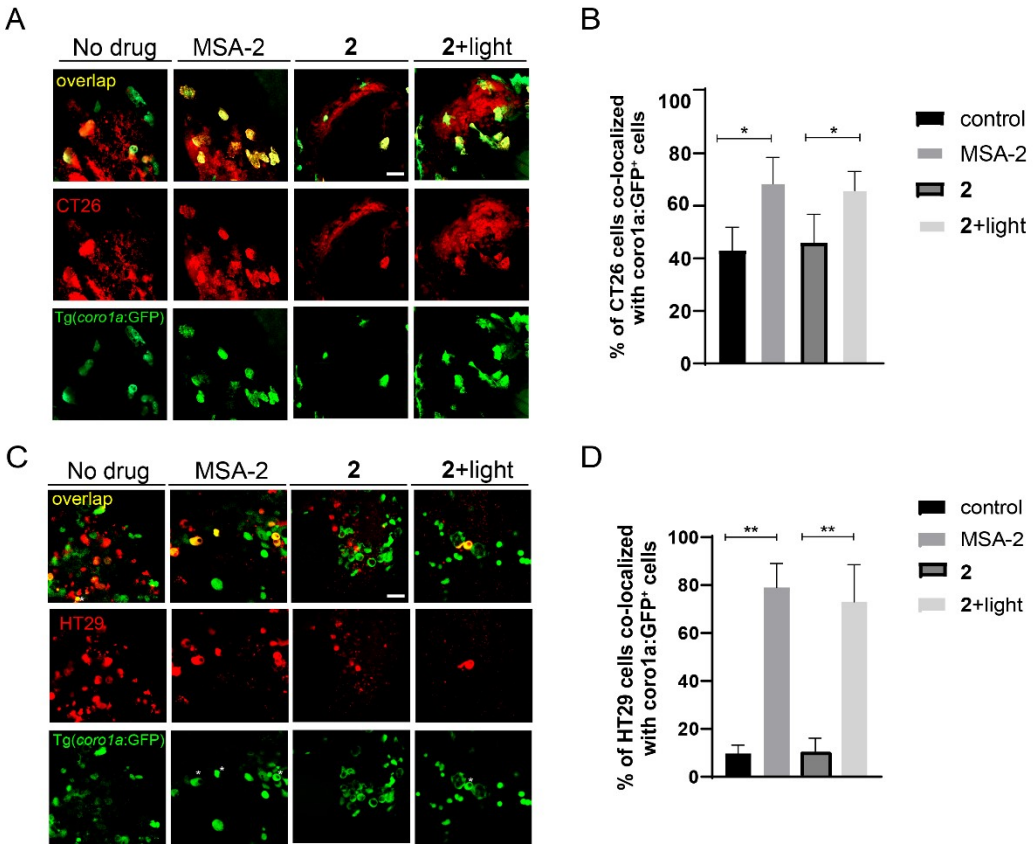

Fig. S8. Photo-triggered co-localization of tumor cells and innate immune cells by **2** in zebrafish xenografts. (A) Representative images of CT26 tumor cells and innate immune cells from Tg (*coro1a*:GFP) zebrafish xenografts at 4 dpi. Compound **2** (10 μM) was added to the embryo medium. (B) Quantification of the percentage of CT26 cells co-localized with innate immune cells at 4 dpi, n = 18 per condition (*$p < 0.05$, t-test). (C) Representative images of HT-29 tumor cells and innate immune cells from Tg (*coro1a*:GFP) zebrafish xenografts at 4 dpi. (D) Quantification of the percentage of HT-29 cells co-localized with innate immune cells at 4 dpi, n = 18 per condition (**$p < 0.01$, t-test). Scale bars: 50 μm; dpi, days post injection.

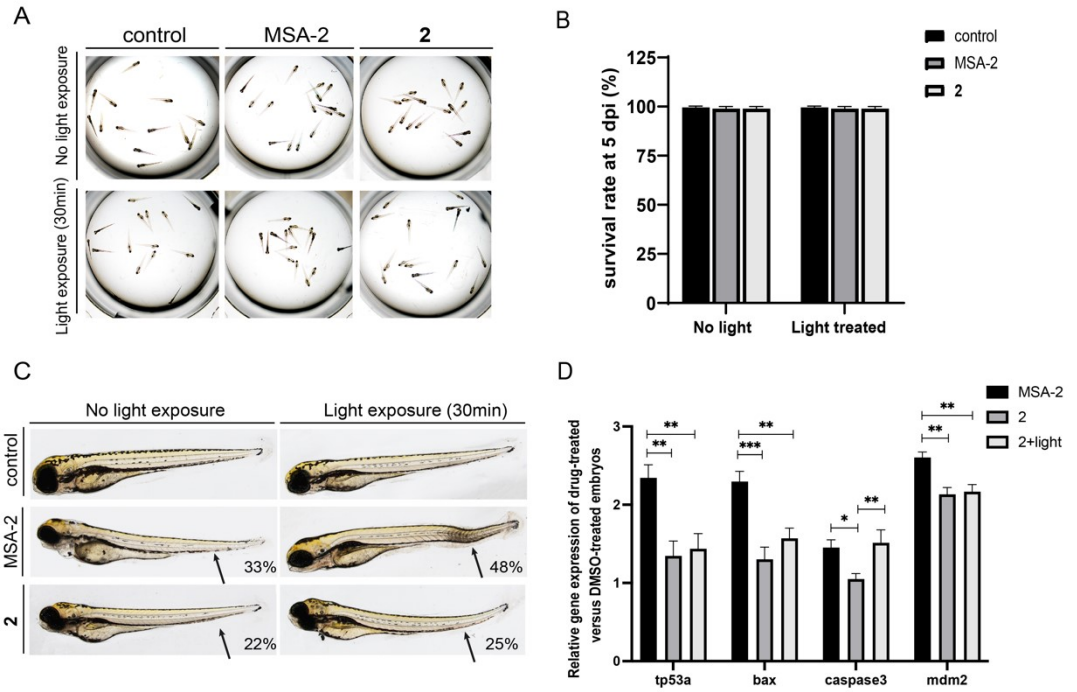

Fig. S9. Embryo survival and tissue toxicity tests after MSA-2 and **2** with and without light activation. (A and B) MSA-2 (40 μM) and **2** (10 μM) with or without light exposure do not affect the embryonic livability after 96 h treatment compared to the control group, n = 15 per group in (B). (C) Representative phenotypes of embryos after MSA-2 and **2** treatments with or without light exposure. The arrows point to the bent and curved tail. Percentages refer to the proportion of embryos with these phenotypes, n = 10-20 embryos per group. (D) Relative expression of the genes involved in apoptotic signaling in embryos after MSA-2 and **2** treatments with or without light irradiation compared to the control DMSO treated embryos. Results are expressed as mean ± SEM from three independent experiments, n = 50 embryos per condition, (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, t-test).

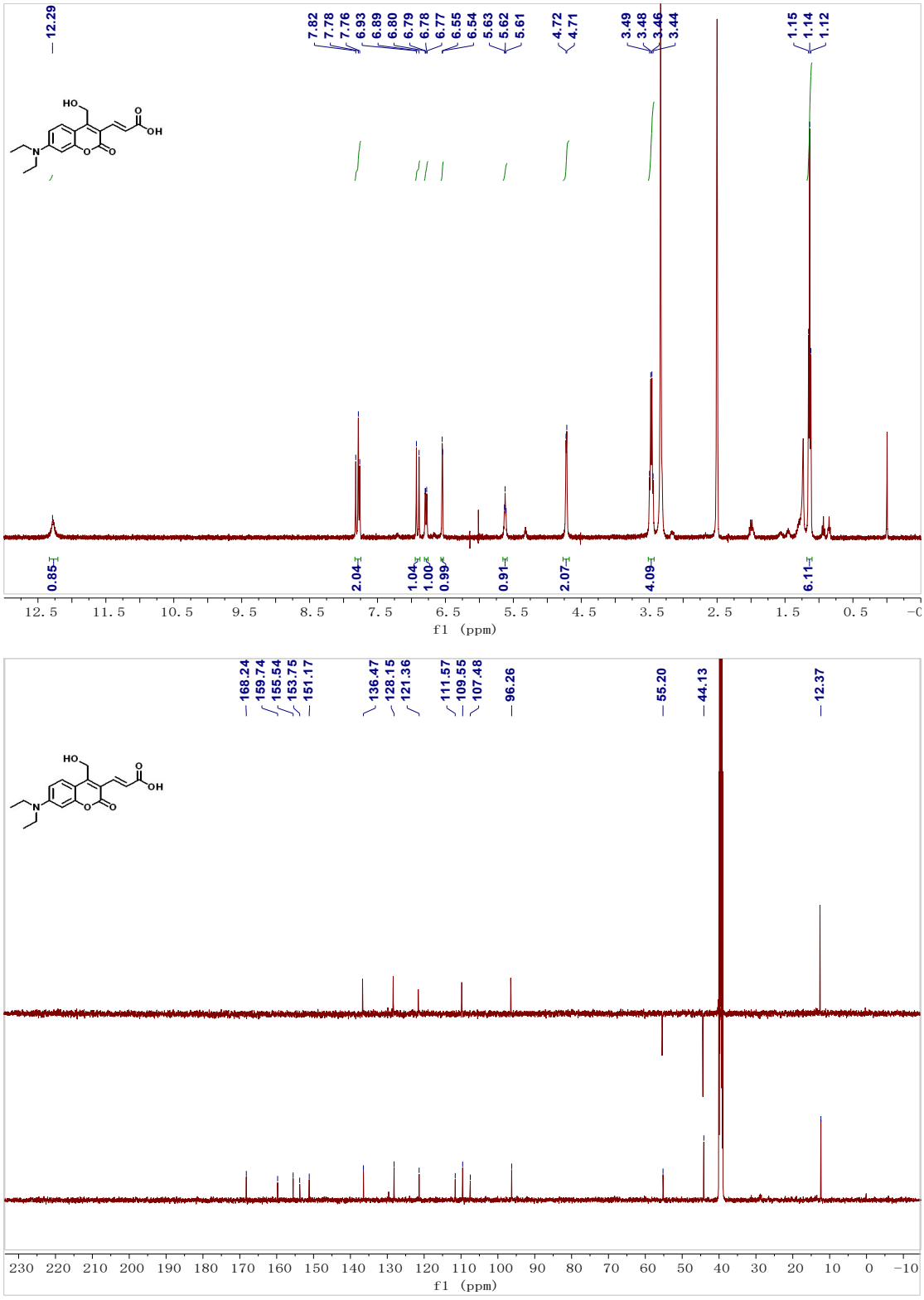

Fig. S10 Copies of ^{1}H and ^{13}C NMR spectra of **5**.

Qualitative Analysis Report

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Acquired Time	7/14/2020 15:51:42	**IRM Calibration Status**	Success
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User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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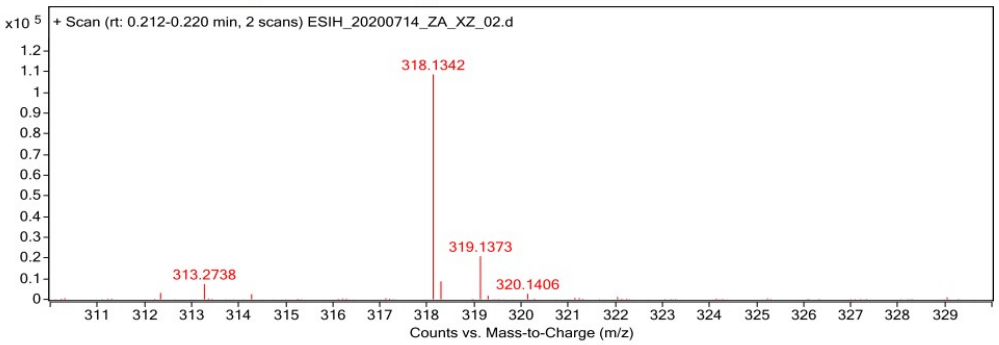

Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
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Fig. S11 Copy of HRMS spectrum of **5**.

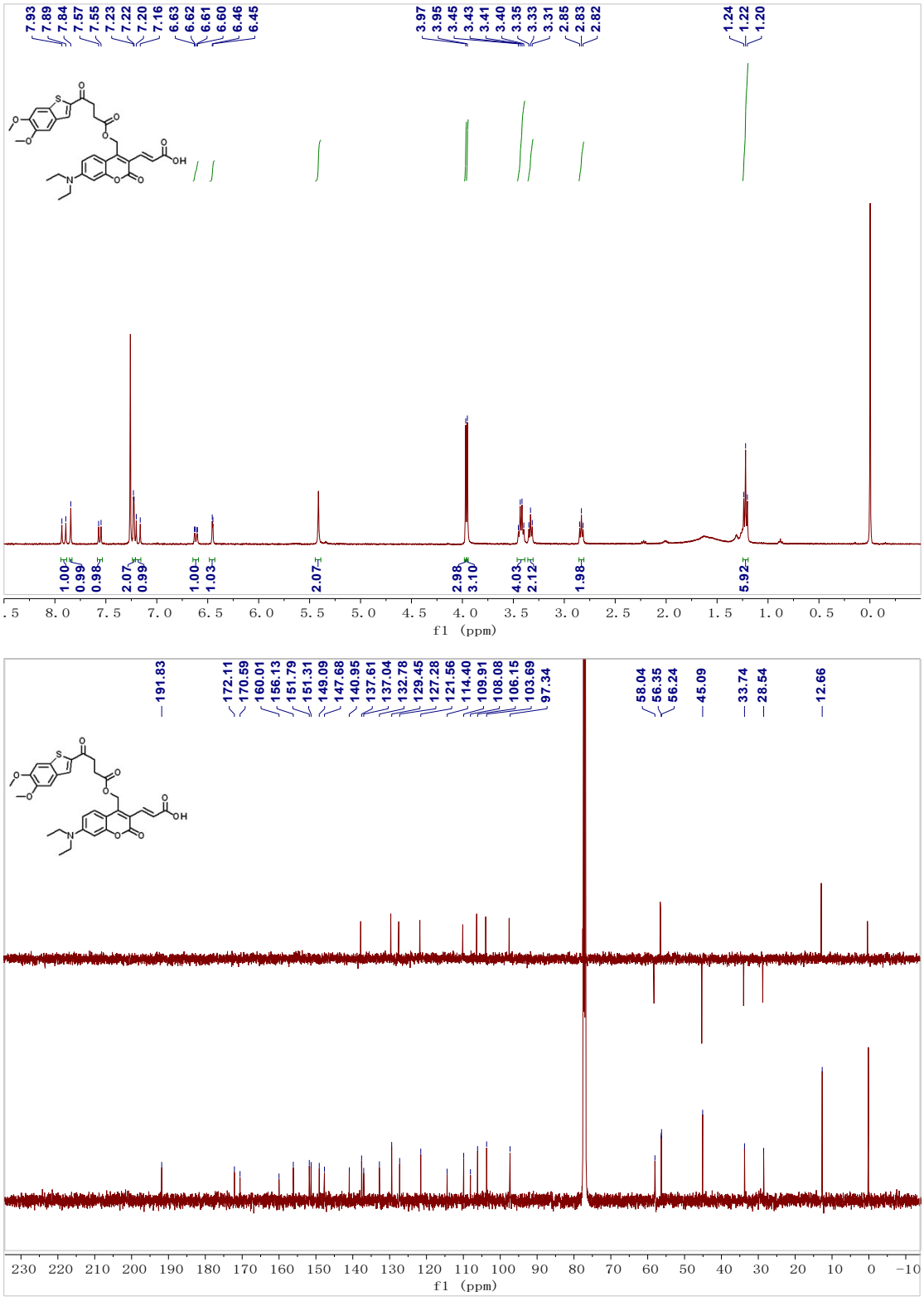

Fig. S12 Copies of ^{1}H and ^{13}C NMR spectra of **1**

Qualitative Analysis Report

Data Filename	ESIH_20200714_ZA_XZ_01.d	**Sample Name**	D5-11980-7
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Agilent G6520 Q-TOF	**Acq Method**	20160322_MS_ESIH_POS_1min.m
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User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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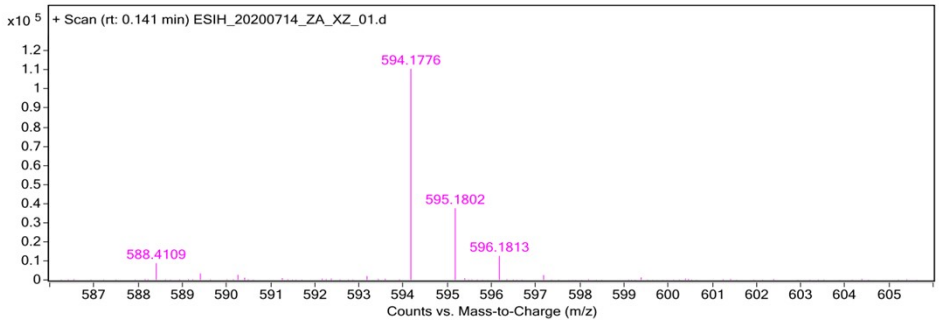

Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
594.1776	594.1792	1.62	2.73	C31 H32 N O9 S	(M+H)+

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Fig. S13 Copy of HRMS spectrum of **1**

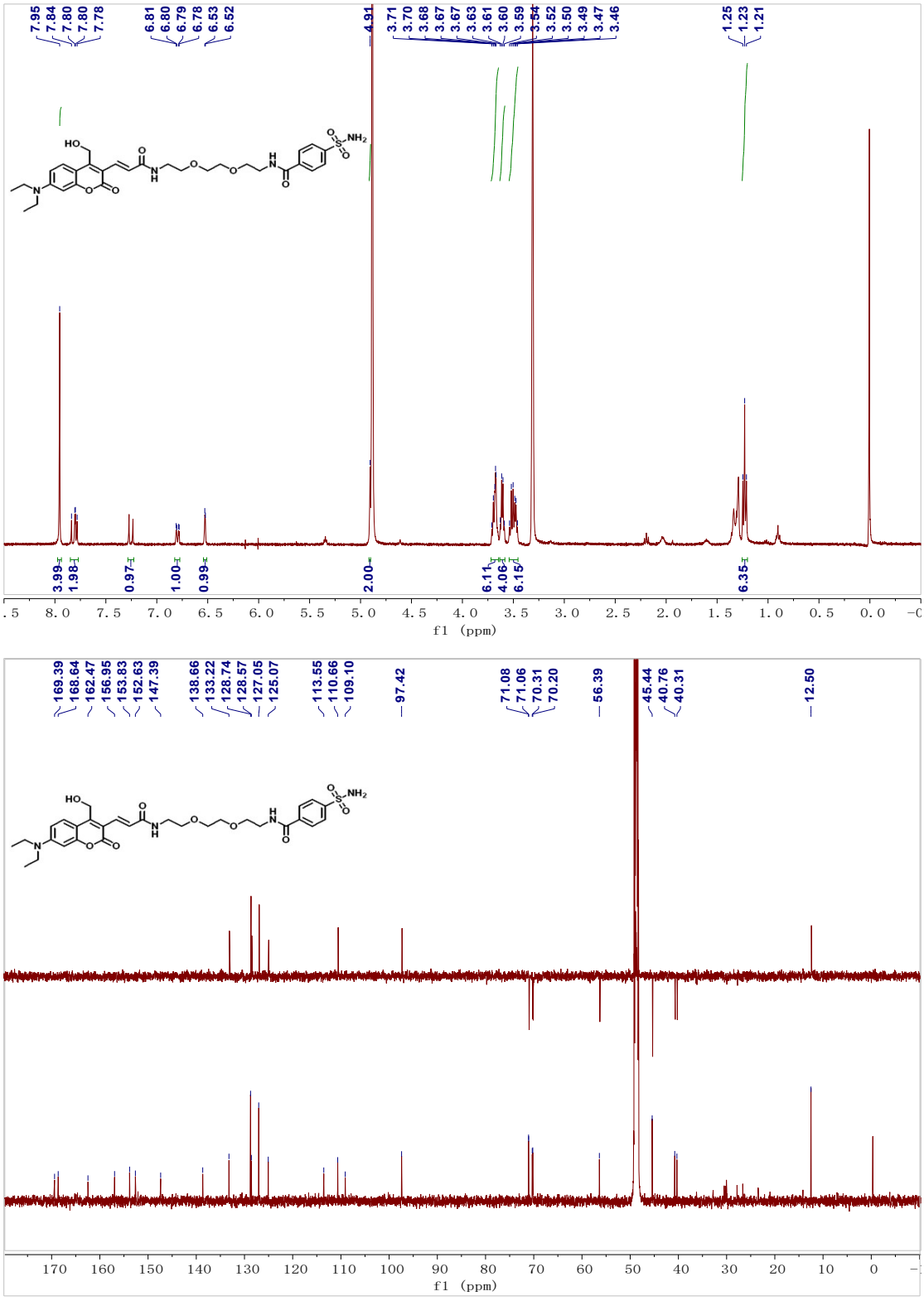

Fig. S14 Copies of ^{1}H and ^{13}C NMR spectra of **9**

Qualitative Analysis Report

Data Filename	ESIH_20200714_ZA_XZ_06.d	**Sample Name**	D5-11980-20
Sample Type	Sample	**Position**	P1-A6
Instrument Name	Agilent G6520 Q-TOF	**Acq Method**	20160322_MS_ESIH_POS_1min.m
Acquired Time	7/14/2020 15:57:33	**IRM Calibration Status**	Success
DA Method	small molecular data analysis method.m	**Comment**	ESIH by ZZY

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
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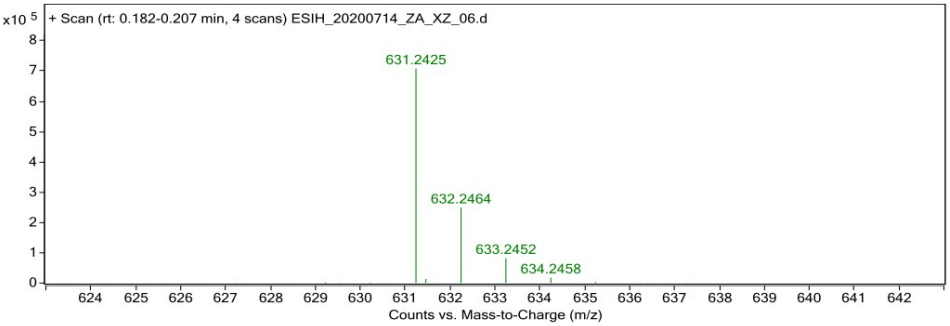

Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
631.2425	631.2432	0.73	1.15	C30 H39 N4 O9 S	(M+H)+

--- End Of Report ---

Fig. S15 Copy of HRMS spectrum of **9**

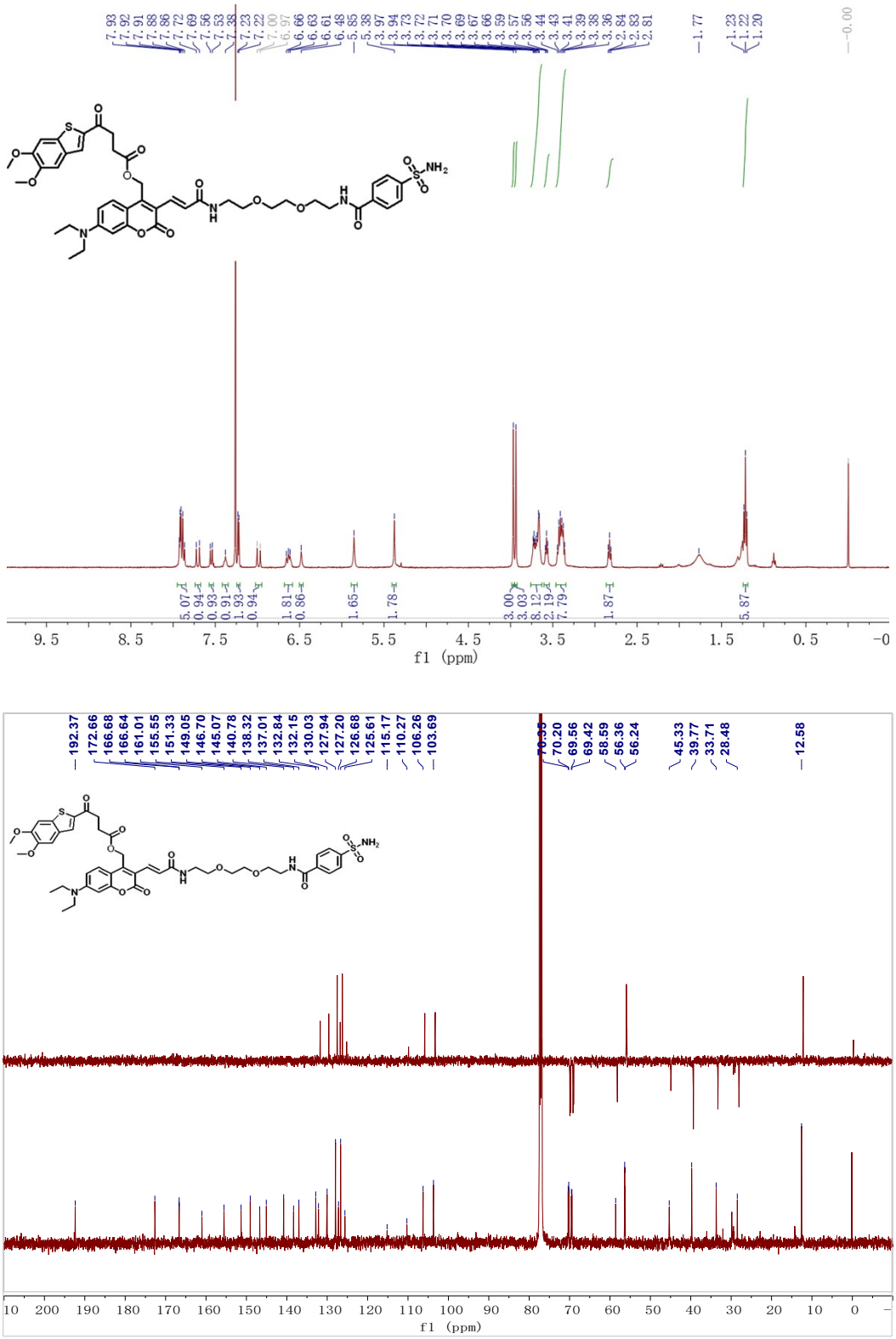

Fig. S16 Copies of ^{1}H and ^{13}C NMR spectra of **2**

Qualitative Analysis Report

Data Filename	ESIH_20200714_ZA_XZ_05.d	**Sample Name**	D5-11980-19
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User Spectra

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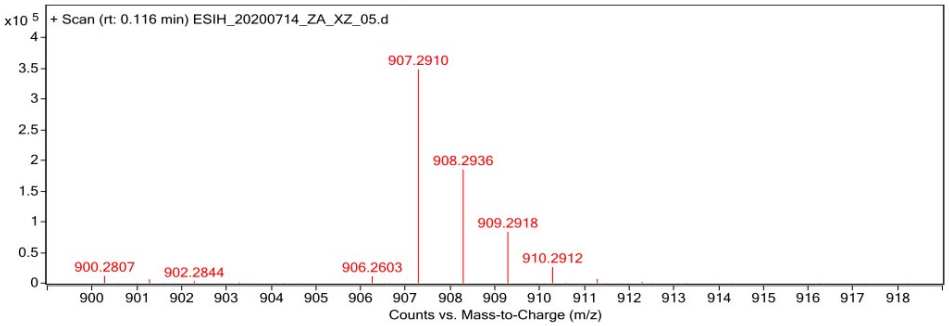

Formula Calculator Results

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--- End Of Report ---

Fig. S17 Copy of HRMS spectrum of **2**