Supporting Information

A bioluminescent probe for hydrogen peroxide imaging in vitro and in vivo

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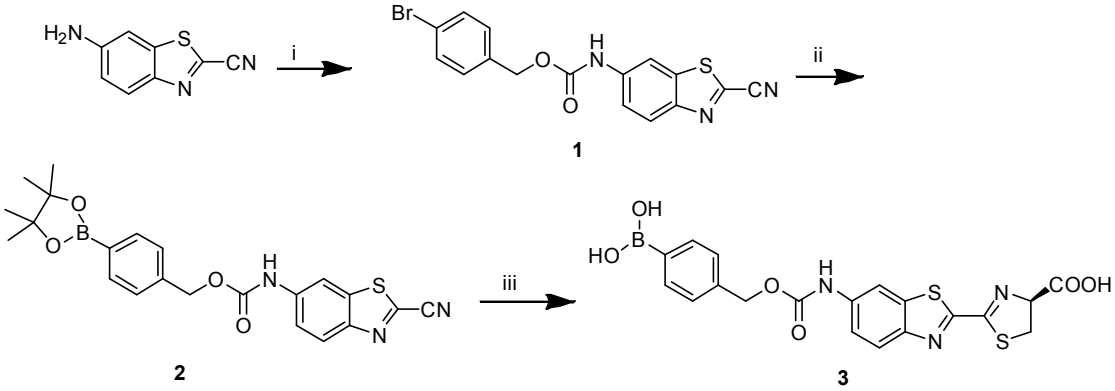
Content

Materials and instruments

All reagents and solvents available from commercial sources were used as received unless otherwise noted. Twice-distilled water was used throughout all experiments. Water used for the bioluminescence studies was doubly distilled and further purified with a Mill-Q filtration system. NMR spectra were obtained in deuterated solvents on Bruker AV-300 or AV-600 spectrometers at the College of Chemistry NMR Facility, Shandong University. All chemical shifts are reported in the standard δ notation of parts per million using the peaks of residual proton and carbon signals of the solvent as internal references. NMR peaks are referred to as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), or broad singlet (br). Coupling constants (J) are reported in hertz. Mass spectra were recorded in ESI^+ mode (70 eV).

Millipore water was used to prepare all aqueous solutions. Measurements for bioluminescent assays were performed in 50 mM Tris buffer, pH 7.4, with 10 mM $MgCl_2$ and 0.1 mM $ZnCl_2$. The bioluminescence spectra were determined with an IVIS Kinetic (Caliper Life Sciences, USA) equipped with a cooled CCD camera was used for bioluminescent imaging.

Synthesis



Scheme S1. Synthesis of probe **3**: (i) bis(trichloromethyl)carbonate (BTC), 4-bromobenzyl alcohol, tetrahydrofuran (THF), from 0 ^oC to room temperature to reflux; (ii) bis(pinacolato)diboron, $Pd(dppf)Cl_2$，potassium acetate (KOAc), 1, 4 -dioxane, N_2, 85 ^oC; (iii) D-cysteine hydrochloride, potassium carbonate (K_2CO_3), $DCM/CH_3OH/H_2O$, N_2, room temperature.

2-(4-bromophenoxy)-N-(2-cyanobenzo[d]thiazol-5-yl)acetamide(1)

5-aminobenzo[*d*]thiazole-2-carbonitrile (102.8 mg, 0.59 mmol) was dissolved in 3 mL dry dimethyl-formamide (DMF), then added the Bis(trichloromethyl)carbonate (BTC) (195.8 mg, 0.66 mmol）solution of THF (1 mL), the mixture was stirred at 0 °C for 0.5 hour, then it was allowed to heat to room temperature for 1 hour. Soon afterwards, 4-Bromobenzyl alcohol (348.0 mg, 1.9 mmol) was added in the mixed solution, reflux 7 hours. The reaction solution was concentrated, the crude product precipitated from the solution as a yellow solid. The yellow solid was dissolved by ethyl acetate, then the organic phase was washed three times with saturated Na_2CO_3 solution，washed twice with brine, dried over magnesium sulfate, and concentrated, The crude material was purified on a silica column (1: 1 petroleum ether: dichloromethane, dry loaded) dried under reduced pressure to afford 200.0 mg of pure product (**1**) as a white powder (78.1%).

^{1}H-NMR (300 MHz, DMSO-*d6*) δ 10.392 (s, 1H), 8.528 (d, *J*=0.9 Hz, 1H), 8.528 (d, *J*=4.8 Hz, 1H), 8.174 (d, *J*=4.2 Hz, 1H), 7.681 (dd, *J*=0.9 Hz, *J*=4.2 Hz, 1H), 7.614 (d, *J*=4.2 Hz, 1H), 7.4212 (d, *J*=4.2 Hz, 2H), 5.187 (s, 2H);

ESI-MS: m/z $[M+H]^+$ 388.3.

N-(2-cyanobenzo[d]thiazol-5-yl)-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetamide (2)

Compound **1** (98.1 mg, 0.25 mmol), Bis(pinacolato)diboron (118.3 mg, 0.47 mmol), Pd(dppf)Cl_2 (11.7 0.0016 mmol) and potassium acetate (121.6 mg, 1.2 mmol) were dissolved in 15mL dry 1, 4–dioxane an N_2 atmosphere. The mixture was stirred at 85 °C for one night before it was allowed to cool to room temperature, diluted with DCM. The organic phase was washed three times with brine, dried over magnesium sulfate, and concentrated. The crude material was purified on a silica column (4: 1 petroleum

ether: ethyl acetate, dry loaded) and dried under reduced pressure to afford 75.0 mg of pure product (**2**) as white powder (68.3%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.398 (s, 1H), 8.532 (d, *J*=1.8 Hz, 1H）, 8.172 (d, *J*=9.0 Hz, 1H), 7.667-7.717 (m, 3H), 7.453 (d, *J*=7.8Hz, 2H), 5.239 (s, 2H), 1.292 (s, 12H);

ESI-MS: m/z $[M+H]^+$ 436.5, $[M+Na]^+$ 458.5.

(S)-2-(5-(2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetamido)benzo[d]thiazol-2-yl)-4,5-dihydrothiazole-4-carboxylic acid(3)

Compound **2** (120.0 mg, 0.28 mmol) was dissolved in 2.5 mL N_2-sparged mixture solution of DCM: CH_3OH (2:3), added D-cysteine hydrochloride (70.0 mg，0.40 mmol) and potassium carbonate (65.0 mg，0.47 mmol) dissolved in 3 mL N_2-sparged DI-H_2O: MeOH (1:1) under an N_2 atmosphere. The reaction was stirred for 10 min away from light. The DCM and MeOH were removed under low pressure, then regulated pH value of the aqueous solution by adding HCl (1M) solution to pH 2-3, a precipitate was formed. Then the precipitate was filtered off, the wet filter cake was recrystallized from methanol, and dried under reduced pressure to afford 94.8 mg of pure product (**3**) as a yellow powder (74.0%).

^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.201 (s, 1H), 10.247 (s, 1H), 8.387 (s, 1H), 8.067-8.082 (m, 3H), 7.811 (d, *J*=7.8Hz, 2H), 7.582 (dd, *J*=9.6Hz, *J*=2.4Hz, 1H), 7.404 (d, *J*=8.4Hz, 2H), 5.427 (t, *J*=9.6Hz, 1H), 5.203 (s, 2H), 3.775 (t, *J*=10.2Hz, 1H), 3.685 (t, *J*=9.6Hz, 1H);

^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.04, 164.35, 158.56, 153.36, 148.14, 138.65, 138.04, 136.48, 134.20 (2C), 130.24, 126.95 (2C), 124.25, 118.86, 110.02, 78.11, 66.01, 34.69;

HRMS m/z calcd. for $C_{19}H_{16}BN_3O_6S_2$ $[M+H]^+$ 457.0683, found 458.0648.

Bioluminescent Assay

Cell bioluminescence imaging of endogenous H_2O_2 for defferent incubation time

Cells were grown in black 96-well plates (4×10^5 cells per well). After a 24-h incubation period, the medium was removed, and cells were treated with 100 μL various concentrations cisplatin(0.02, 0.04, 0.40, 0.80, 1.60 μM). After incubating for 24, 12, 6, 4 h in the incubator, the medium removed, 50 μL probe 3 (100 μM) was added to each well and the luciferase activity was measured 5 min later using a Xenogen IVIS Spectrum imaging system. Luminescent signal (photons per second) for each well was measured and plotted as average values. These results demonstrated when cells were incubated with cisplatin for 12 h, the endogenous concentration of H_2O_2 could reach the maximal value (Figure S1).

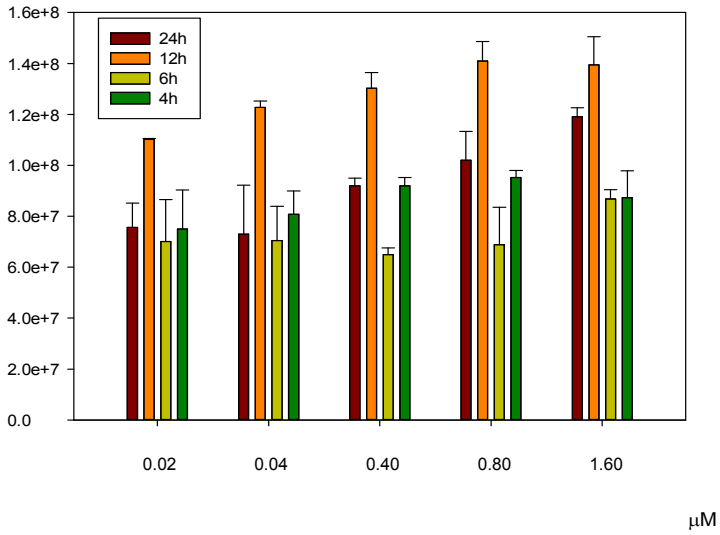


Figure S1: Quantification of bioluminescence imaging of ES-2-luc cells incubated with probe **3** and various concentrations cisplatin for different incubation time.

Cell bioluminescence imaging of endogenous H_2O_2 at different measure time point

Cells were grown in black 96-well plates (4×10^5 cells per well). After a 24 h incubation period, the was removed, and cells were treated with 100 μL various concentrations of cisplatin (0, 6.25, 12.5, 25, 50 and 100 μM). After 12 h incubation, the medium was removed, then 50 μL probe **3** (100 μM) was to each well and the luciferase activity was measured at 1, 2, 3, 4, 7, 8, 10, 12, 15, 20, 25 and 30 min by

using a Xenogen IVIS Spectrum imaging system. Luminescent signals (photons per second) for each well were measured and plotted as average values. These results indicated that the peak of luminescent signal between 4 and 7 min (Figure S2). Therefore, a time point at 5 min was selected for maximizing the experimental signals and minimizing the possible background.

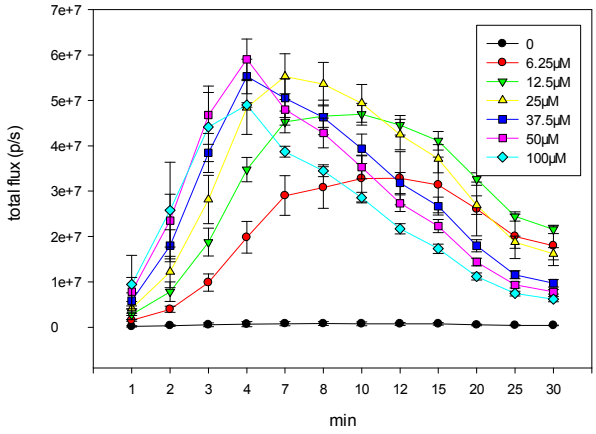


Figure S2: Quantification of bioluminescence imaging of ES-2-luc cells incubated with probe **3** and cisplatin measured at different time points

Cell bioluminescence imaging of endogenous H_2O_2 with or without free radical scavenging agents

Cells were grown in black 96-well plates (4×10^5 cells per well). After a 24h incubation period, the medium was removed, and cells were treated with 100 μL of 33 μM cisplatin, After 12 h incubation, 100 μL medium or NAC (1 mM, dissolved by medium) or sodium ascorbate (1 mM, dissolved by medium) were added individually and incubated for 30 min. The medium was then removed, and 50 μL probe **3** (100 μM) was added to each well and the luciferase activity was measured 5 min later using a Xenogen IVIS Spectrum imaging system. Luminescent signal (photons per second) for each well was measured and plotted as average values (Figure S3). These results suggested that the probe's ability is identifying H_2O_2 indeed.

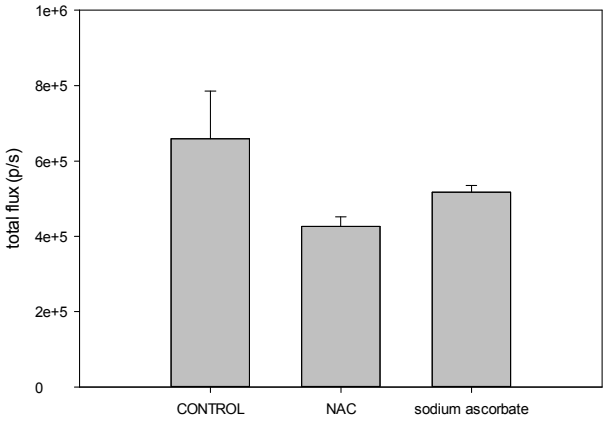


Figure S3: Quantification of bioluminescence imaging of the endogenous H_2O_2 activity with probe **3** in ES-2-luc cells incubated with medium or NAC or sodium ascorbate

^{1}H-NMR, ^{13}C-NMR and ESI-MS spectra of compounds

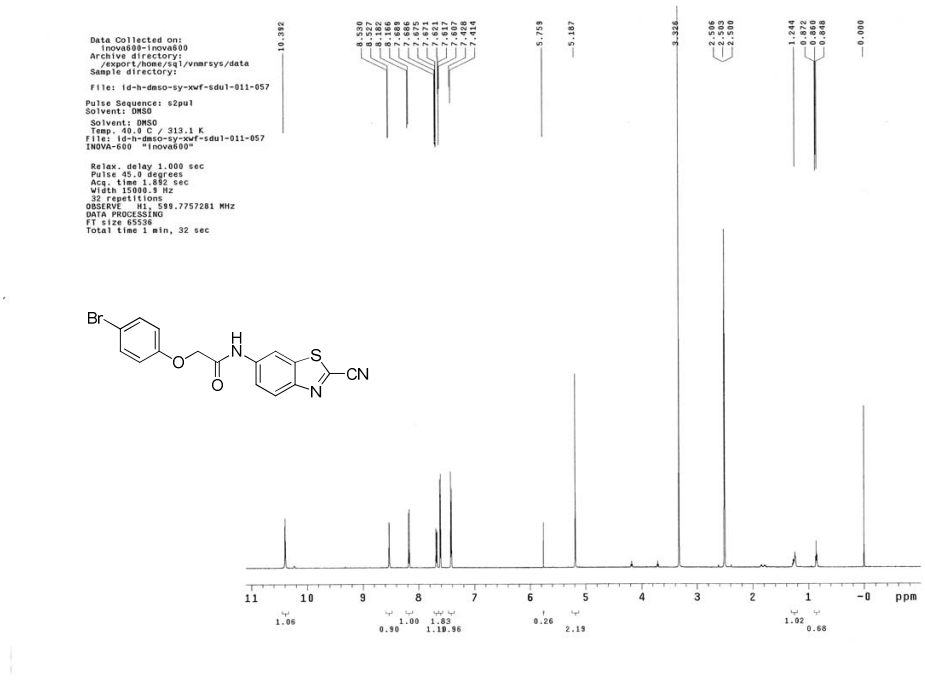


Figure S4: ^{1}H-NMR spectra of compound **1**

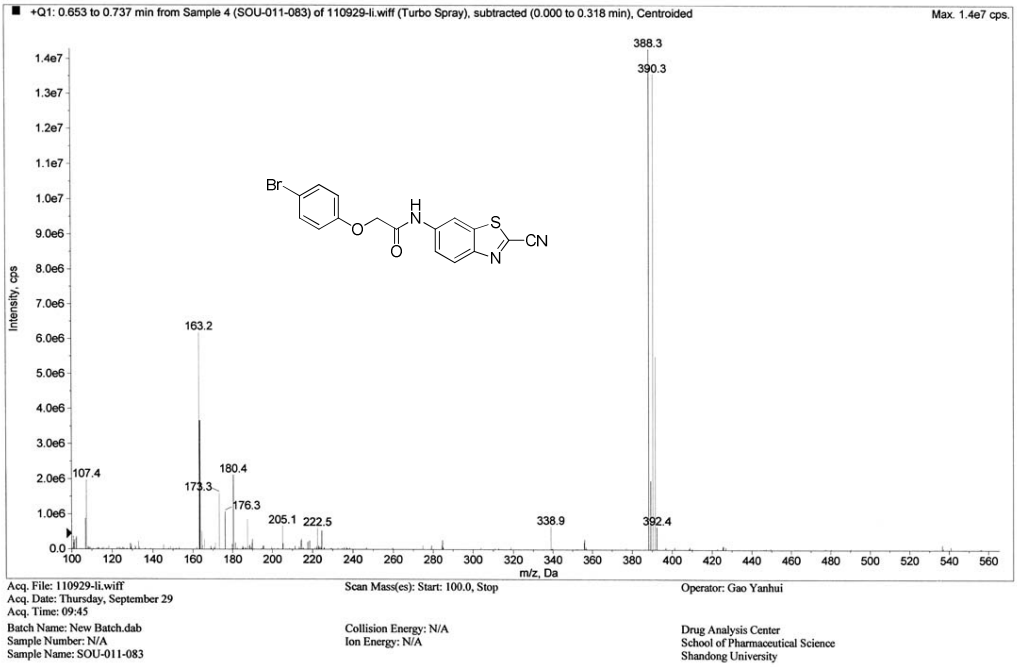


Figure S5: ESI-MS spectra of compound **1**

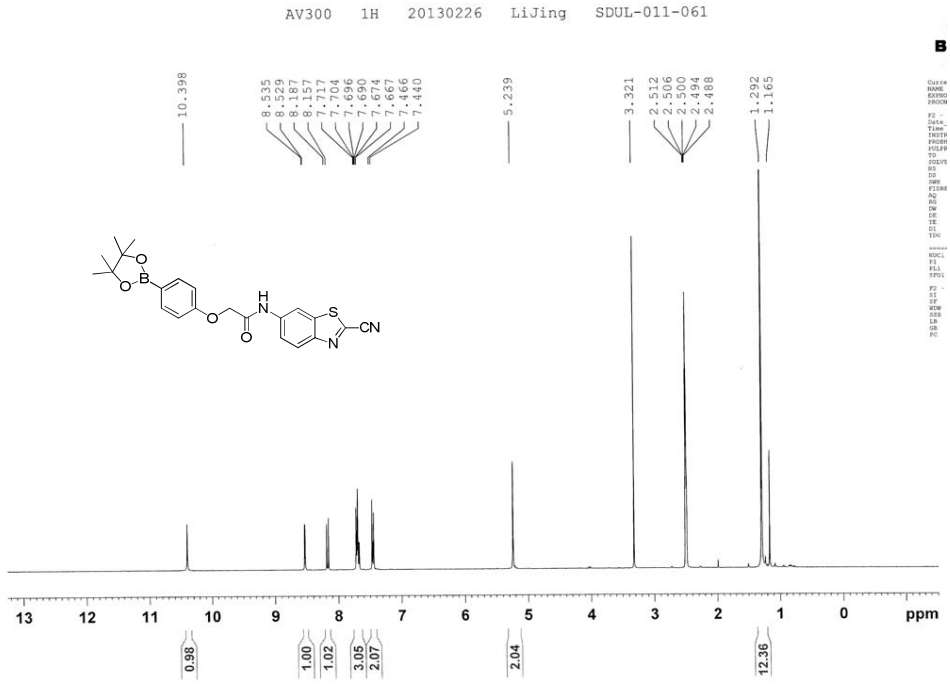


Figure S6: ^{1}H-NMR spectra of compound **2**

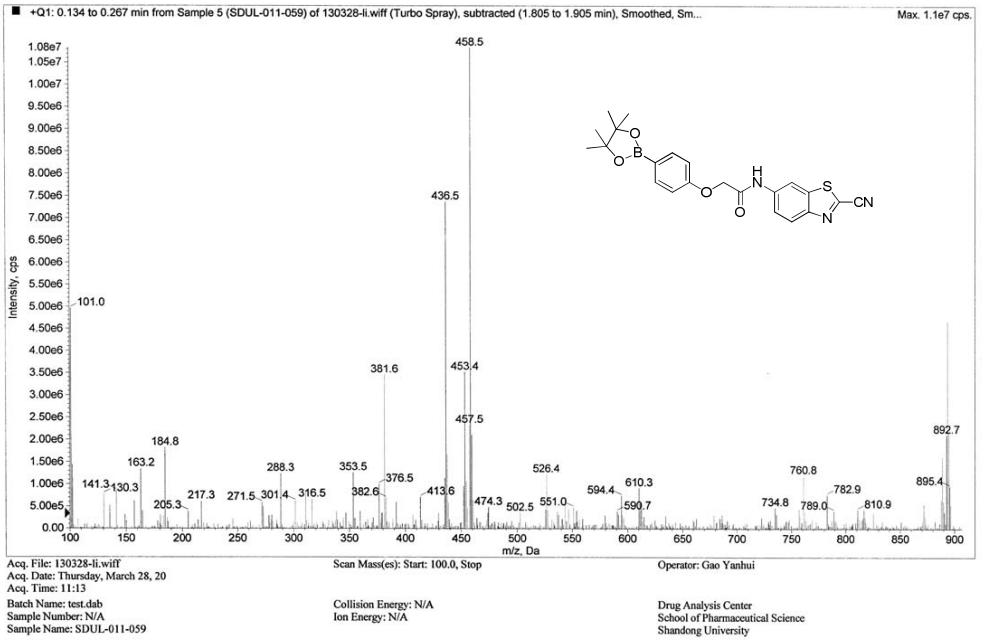


Figure S7: ESI-MS spectra of compound **2**

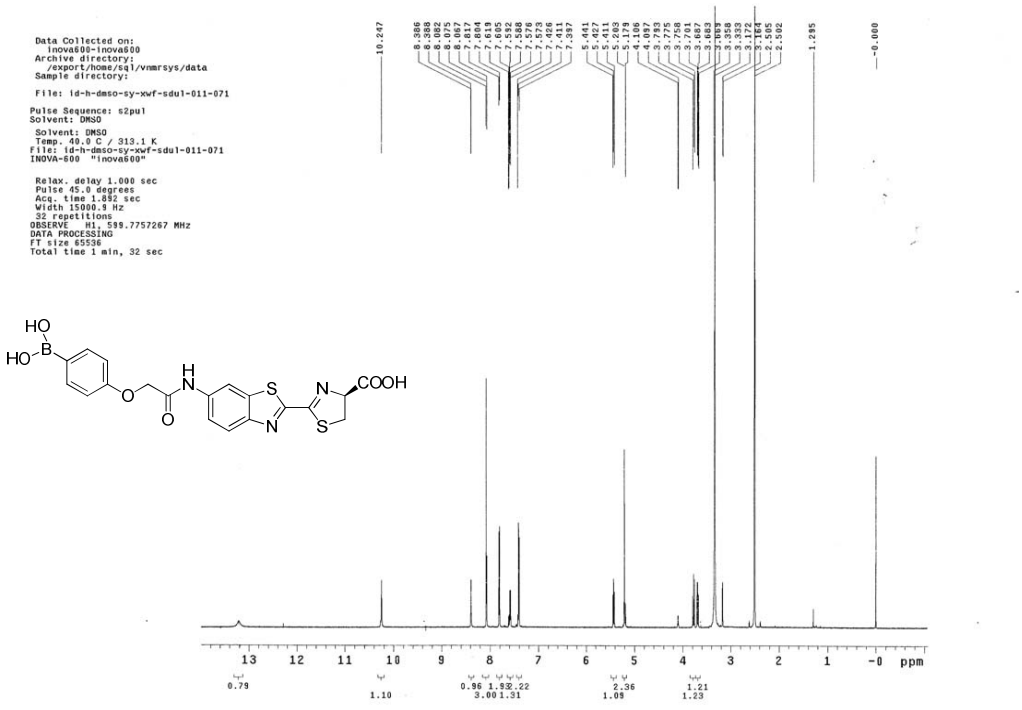


Figure S8: ^{1}H-NMR spectra of compound **3**

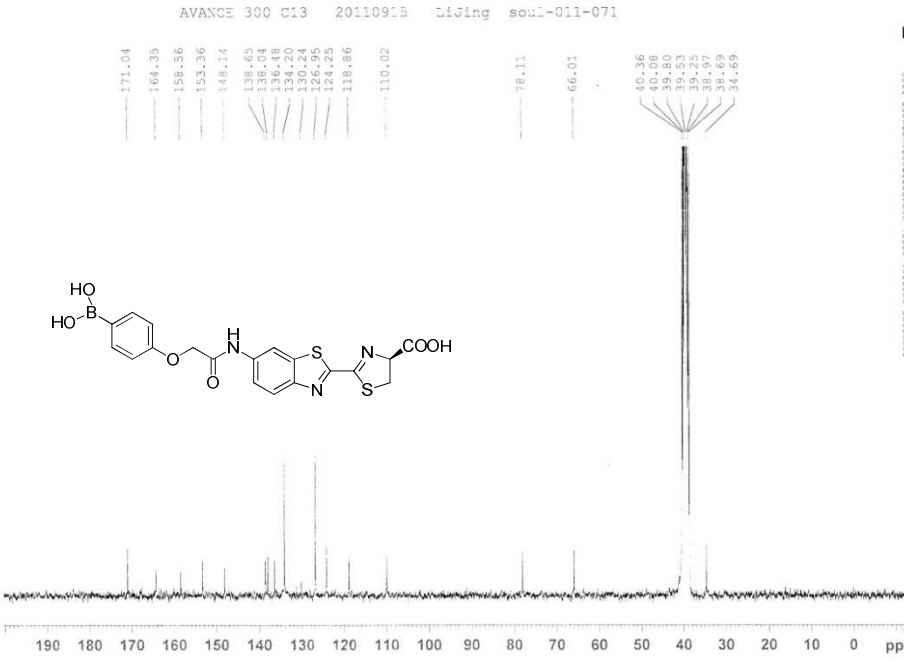


Figure S9: ^{13}C-NMR spectra of compound **3**

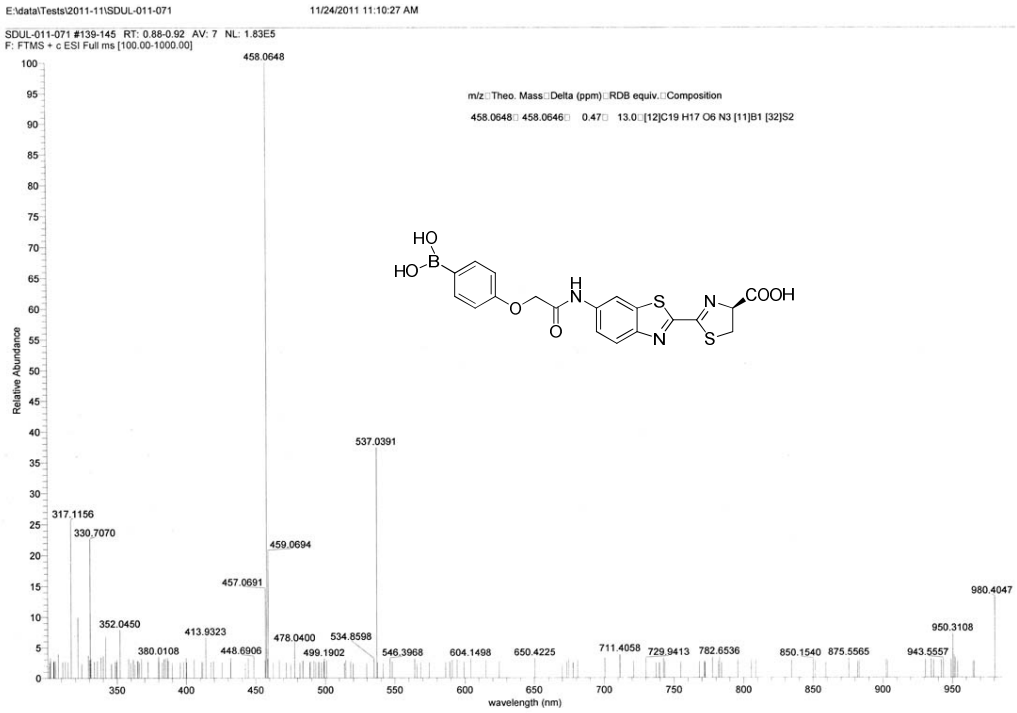


Figure S10: ESI-MS spectra of compound **3**