Electronic Supplementary Information for

A lipid droplet-targetable and biothiol-sensitive fluorescent probe for diagnosis of cancer cells/tissues

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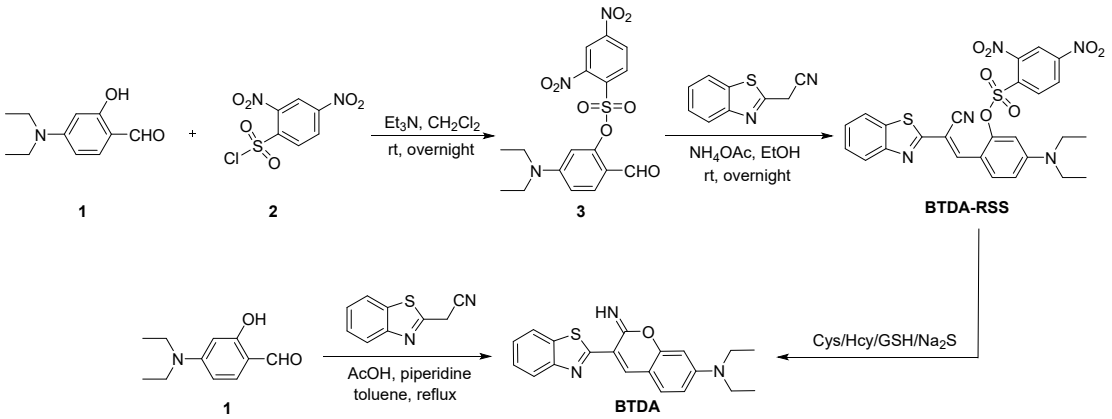
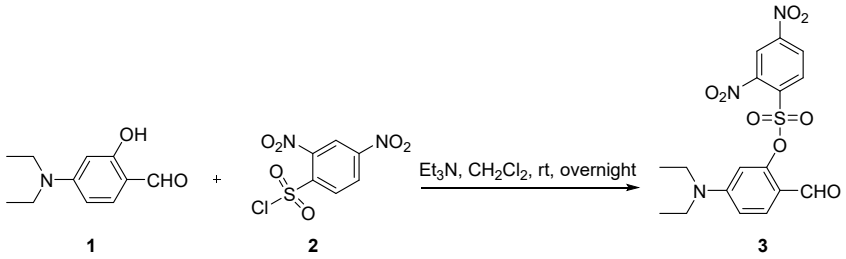
Contents

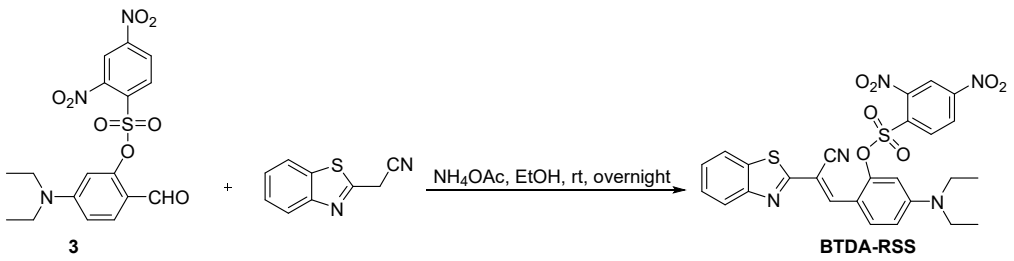
Experimental section



Scheme S1 Synthetic route to **BTDA-RSS** and **BTDA**.

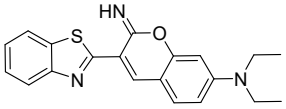


Compound **1** (20 mg, 0.10 mmol) was dissolved in DCM (1 mL) at 0 °C, and compound **2** (40 mg, 0.15 mmol) was added followed by Et_3N (24 μL, 0.16 mmol). The resulting solution was stirred at room temperature for overnight. And then the solvent was concentrated in vacuo, the residue was purified using silica gel chromatography (PE/EtOAc, 5:1, *v*/*v*) to give compound **3** as a red solid (25 mg, 60 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (s, 1H), 8.66 (d, J = 2.4 Hz, 1H), 8.54 (dd, J = 8.8, 2.4 Hz, 1H), 8.36 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 9.2 Hz, 1H), 6.63 (dd, J = 8.8, 2.4 Hz, 1H), 6.52 (d, J = 2.8 Hz, 1H), 3.43 (q, J = 7.2 Hz, 4H), 1.22 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.5, 153.2, 151.8, 151.0, 149.0, 134.0, 133.8, 132.6, 126.7, 120.4, 116.3, 110.3, 105.0, 45.2, 12.4. HRMS (ESI-TOF): calcd. for $C_{17}H_{18}N_3O_8S$ $[M + H]^+$ 424.0809; found 424.0819.



A mixture of compound **3** (100 mg, 0.24 mmol), benzothiazole-2-acetonitrile (47

mg, 0.27 mmol), and ammonium acetate (21 mg, 0.27 mmol) in EtOH (5 mL) was stirred at room temperature for overnight. And then the solvent was concentrated in vacuo, the residue was purified using silica gel chromatography (PE/EtOAc, 5:1, *v*/*v*) to give compound **BTDA-RSS** as a red solid (22 mg, 30 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (dd, J = 8.4, 2.0 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 8.04 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 6.72 (dd, J = 9.2, 2.0 Hz, 1H), 6.68 (d, J = 2.4 Hz, 1H), 3.49 (q, J = 7.2 Hz, 4H), 1.28 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 153.4, 152.0, 151.1, 150.6, 149.0, 139.4, 134.7, 133.7, 133.6, 130.5, 127.4, 126.7, 126.1, 123.0, 122.0, 120.9, 117.2, 111.9, 111.3, 106.8, 100.0, 45.3, 12.6. HRMS (ESI-TOF): calcd. for $C_{26}H_{22}N_5O_7S_2$ $[M + H]^+$ 580.0955; found 580.0950.



BTDA

A mixture of compound **1** (195 mg, 1 mmol), benzothiazole-2-acetonitrile (349 mg, 2.0 mmol) piperidine (300 μL, 3.2 mmol), acetic acid (300 μL, 5.2 mmol) in 50 mL toluene was refluxed under nitrogen atmosphere for 16 h. After cooling to room temperature, the mixture was washed with brine and extracted with dichloromethane (3 × 15 mL). The organic layer was dried over anhydrous $MgSO_4$. After concentrated, ethyl ether/dichloromethane (30:1, v/v) was added and **BTDA** was precipitated and collected as a red solid (280 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.8 Hz, 2H), 7.36 (t, J = 6.8 Hz, 1H), 6.67 (dd, J = 8.8, 2.4 Hz, 1H), 6.56 (d, J = 2.4 Hz, 1H), 3.45 (q, J = 7.2 Hz, 4H), 1.25 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 161.2, 157.1, 152.7, 152.2, 142.1, 136.3, 130.9, 126.2, 124.5, 122.2, 121.7, 112.4, 110.1, 108.7, 97.0, 45.2, 12.7. HRMS (ESI-TOF): calcd. for $C_{20}H_{20}N_3OS$ $[M + H]^+$ 350.1322; found 350.1321.

Cell culture and cell cytotoxicity assay

All the cells lines were kindly provided by Modern Research Center for Tradition Chinese (Shanxi University, Taiyuan, China). HeLa, 4T1, A549, MPC5, PC12 and TM3 cells were cultured in RPMI1640 or DEME medium supplemented with 10 % fetal bovine serum and 1% antibiotics at 37 ℃ in a 5 % CO_2 atmosphere. The cell

cytotoxicity of **BTDA-RSS** to living HeLa cells was performed by a standard *CCK-8* assay (cell counting kit-8). About 1×10^4 cells/well in 200 μL cell culture medium were seeded in 96-well microplate and then the medium was replaced with fresh medium that containing **BTDA-RSS** with various concentrations of 0 μM、0.5 μM、1 μM、2.5 μM、5 μM、7.5 μM、10 μM for 24 h, respectively. After washing the cells with fresh medium three times, 20 μL *CCK-8* in 180 μL PBS was loaded to each well for another 4 h. Then each well was analysed with an ELISA microplate reader and the absorbance was detected at 450 nm. The cell viability was expressed as relative to the control cells taken as 100 % metabolic activity.

Fluorescent imaging in living Cells/tissues

For colocalization experiments: HeLa cells were co-incubated with **BTDA-RSS** (5 μM) and Nile Red (0.3 μM), LB-NIR or MTDR (1 μM) for 30 min, respectively. The fluorescence images were captured using a CLSM. **BTDA-RSS**: λ_{ex} = 488 nm, λ_{em} = 500 - 600 nm; Nile Red: λ_{ex} = 561 nm, λ_{em} = 600 - 650 nm; LB-NIR: λ_{ex} = 633 nm, λ_{em} = 650 - 750 nm; MTDR: λ_{ex} = 633 nm, λ_{em} = 650 - 750 nm.

*For biothiols-sensitive ability of **BTDA-RSS** in living cells*: HeLa cells were incubated with **BTDA-RSS** (5 μM) for 30 min. HeLa cells were first incubated with NEM (1 mM) for 30 min, then treated with Cys (100 μM), Hcy (100 μM), GSH (100 μM) or Na_2S (100 μM) respectively, for another 30 min.

*For visualization of cancer cells using **BTDA-RSS***: Living cancer cells (HeLa, 4T1 and A549) and living normal cells (MPC5, PC12 and TM3) were incubated with **BTDA-RSS** (5 μM) for 30 min, respectively.

*For visualization of animal cancer tissues using **BTDA-RSS***: Tumor-bearing mice were prepared by subcutaneous injection of HeLa cells into the right axillae of nude mice for 14 days. Then, the normal organs (heart, spleen, liver, lung and kidney) and tumor were isolated from the mice, and sectioned as 5 μm thicknesses. These tissue slices were incubated with **BTDA-RSS** (20 μM) for 30 min.

*For visualization of human cancer tissues using **BTDA-RSS***: The harvested surgical specimens of patients, including two benign tissues (including thyroid and breast), and their malignant tissues, were cryosectioned as 5 μm thicknesses. For living human tissue slices harvested surgical specimens of patients were kindly provided by

Department of Pathology in Shanxi Provincial People's Hospital, and stained with POTA-OH (20 μM) for 30 min, respectively. Informed consent was obtained for any experiment conducted on human subjects.

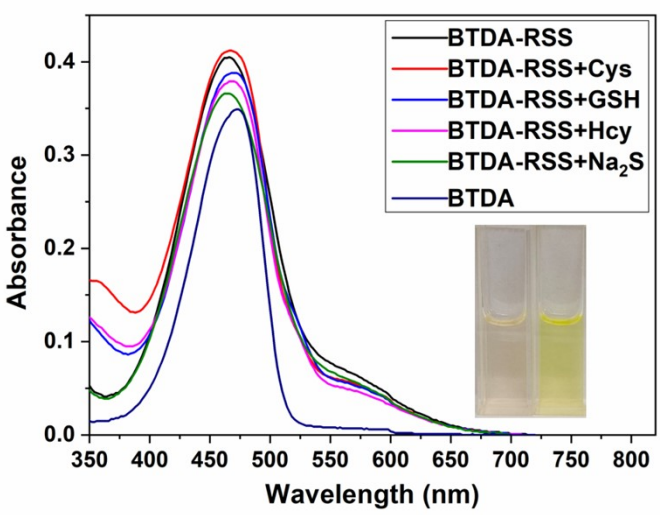


Fig. S1 UV−vis spectral changes of **BTDA-RSS** (2.5 μM) with 20 μM of Hcy, GSH, Cys and Na_2S in PBS/DMSO (1/1, v/v, pH 7.4). Inset: the color of BTDA-RSS solution in the absence or presence of biothiols under a nature light.

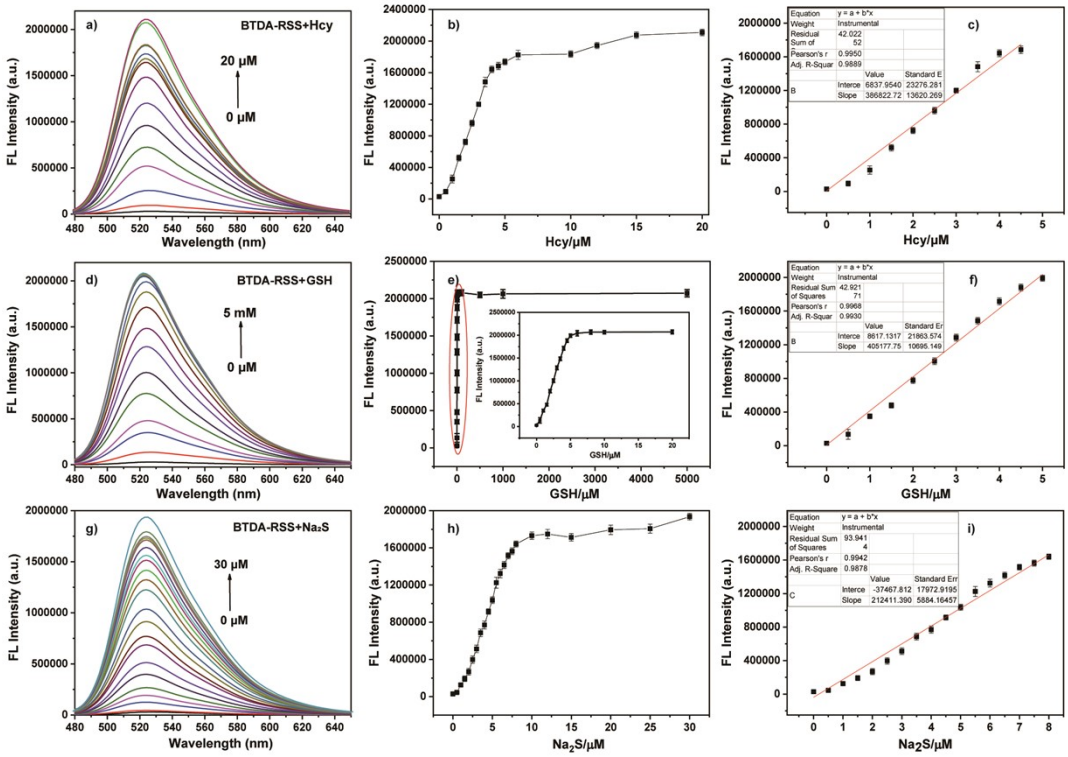


Fig. S2 Fluorescence emission spectra of **BTDA-RSS** (2.5 μM) upon addition of Hcy (0-20 μM) (a), GSH (0-5 mM) (d) and Na_2S (0-30 μM) (g) in PBS/DMSO (1/1, v/v, pH 7.4), excited at 458 nm; Fluorescence intensity ($F_{528\ nm}$) vs the concentrations of Hcy (b), GSH (e) and Na_2S (h) curves; The linear relationships over the concentrations of Hcy from 0 to 4.5 μM (c), GSH from 0 to 5 μM (f) and Na_2S 0 to 8 μM (i).

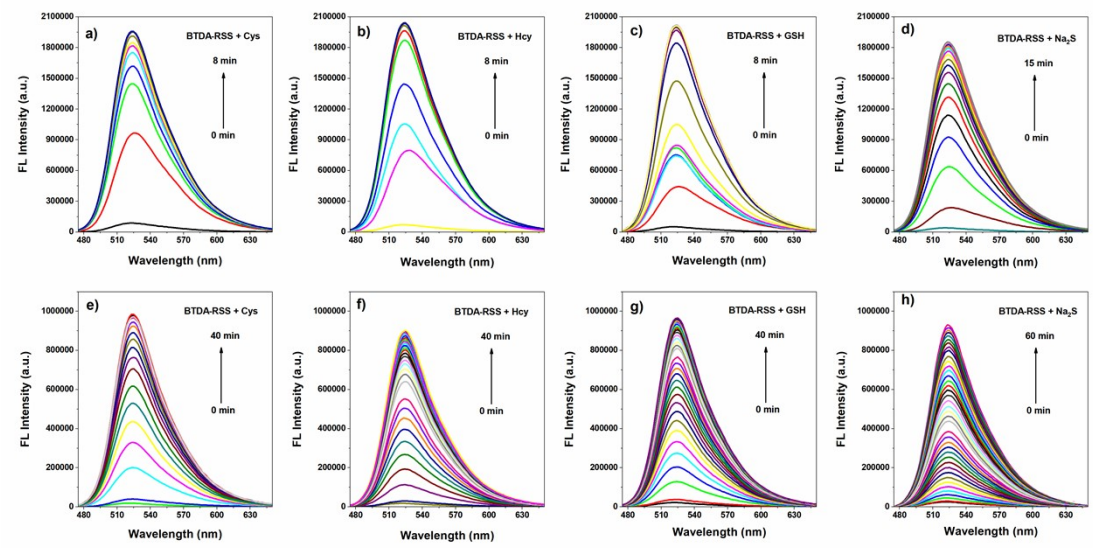


Fig. S3 Fluorescence emission spectra of **BTDA-RSS** (2.5 μM) upon addition of Cys (0-20 μM), Hcy (0-20 μM), GSH (0-5 mM), Na_2S (0-30 μM) in PBS/DMSO (6/4, v/v, pH 7.4) (a-d) and PBS/DMSO (8/2, v/v, pH 7.4) (e-h), excited at 458 nm.

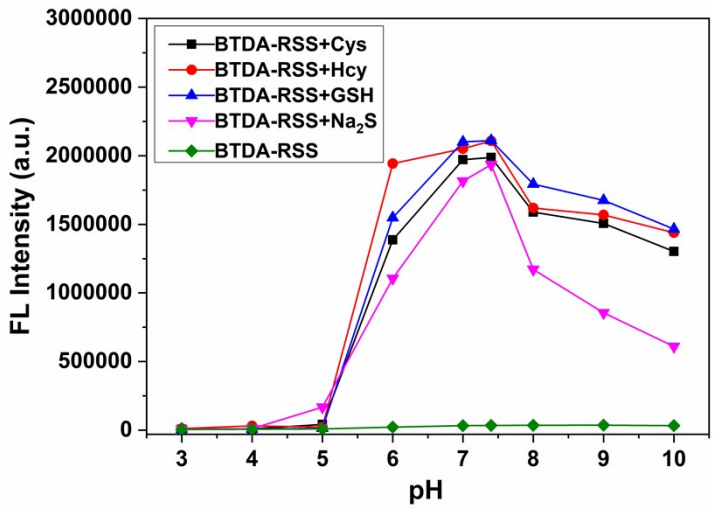
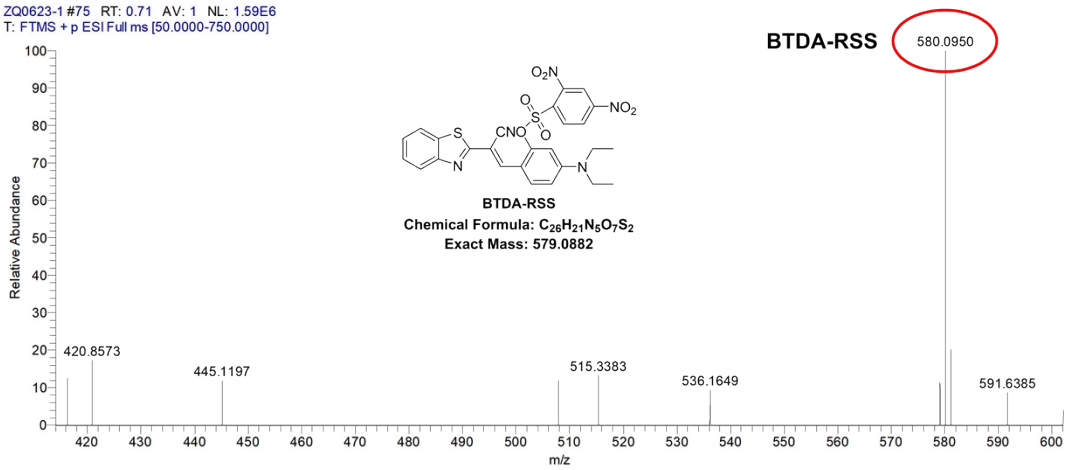


Fig. S4 Fluorescence emission spectra of **BTDA-RSS** upon addition of Cys (20 μM), Hcy (20 μM), GSH (20 μM), Na_2S (30 μM) under different pH conditions.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BTDA-RSS
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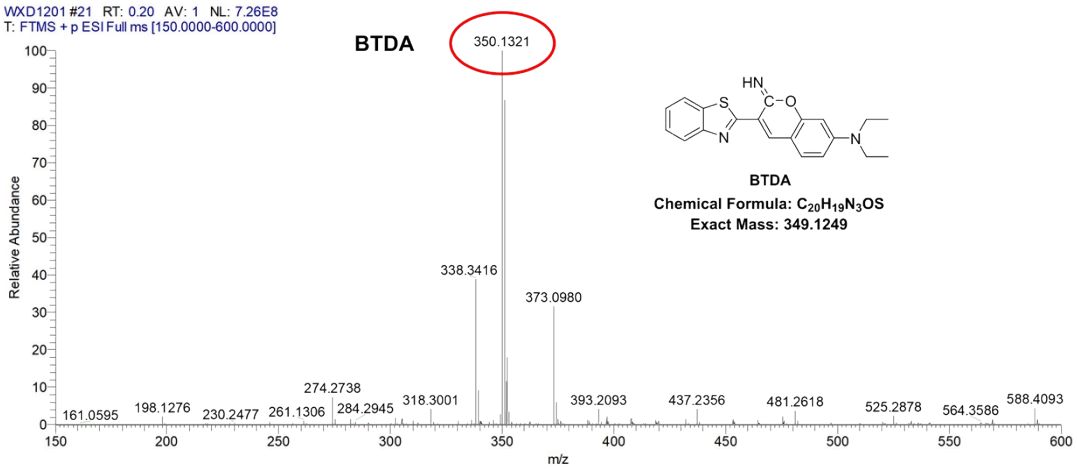
536.1649
591.6385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T: FTMS + p ESI Full ms [150.0000-600.0000]
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BTDA
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Relative Abundance
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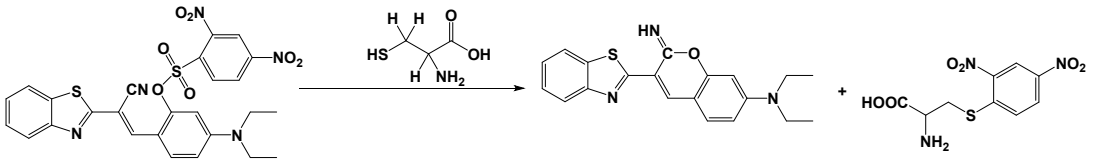
BTDA-RSS+Cys



Chemical Formula: $C_{26}H_{21}N_5O_7S_2$
Exact Mass=579.0882

Chemical Formula: $C_{20}H_{19}N_3OS$
Exact Mass=349.1249

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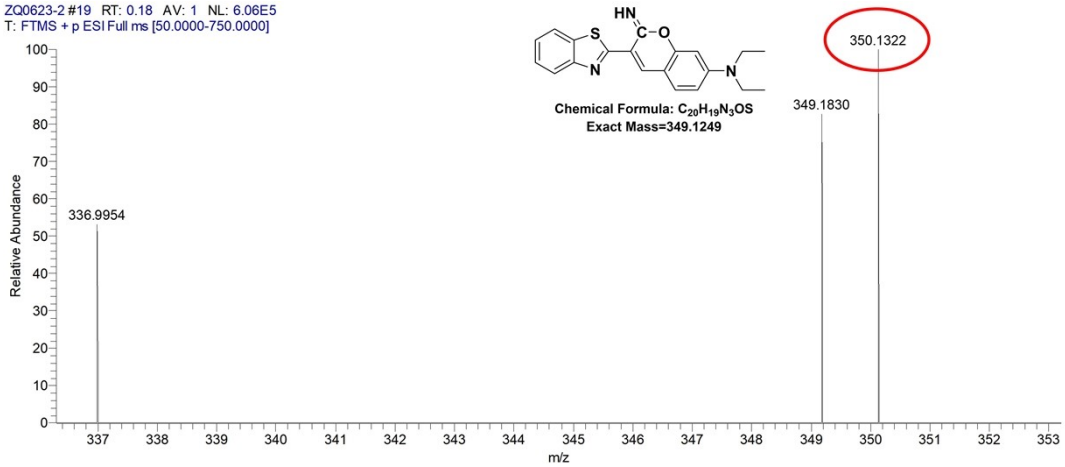
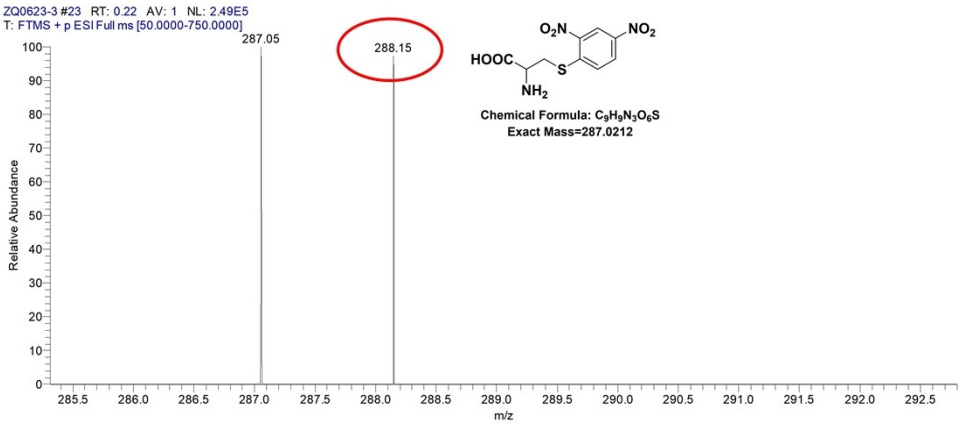
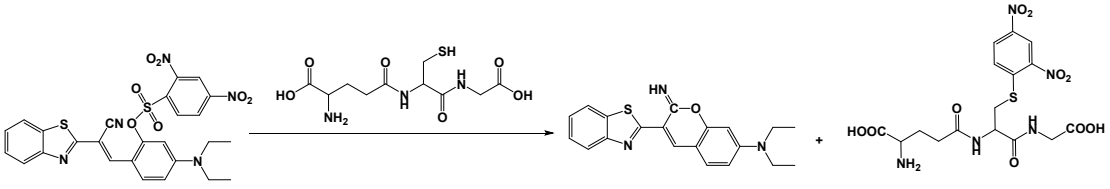




BTDA-RSS+GSH



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Exact Mass=579.0882

Chemical Formula: $C_{20}H_{19}N_3OS$
Exact Mass=349.1249

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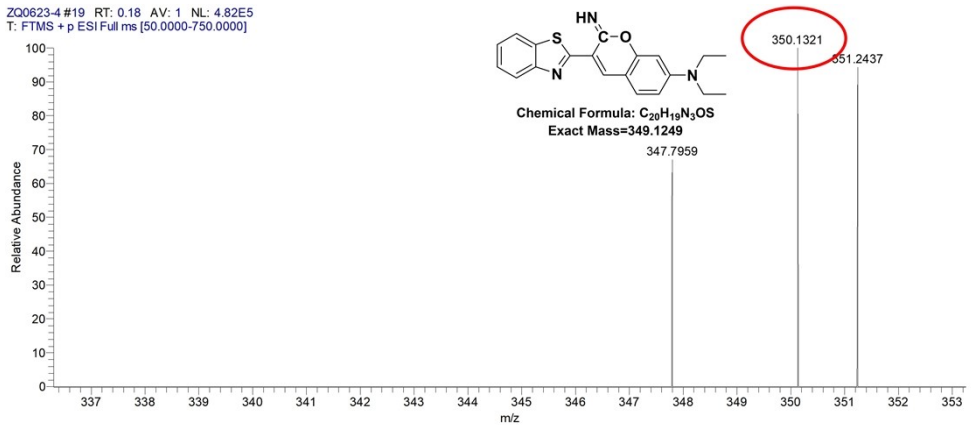
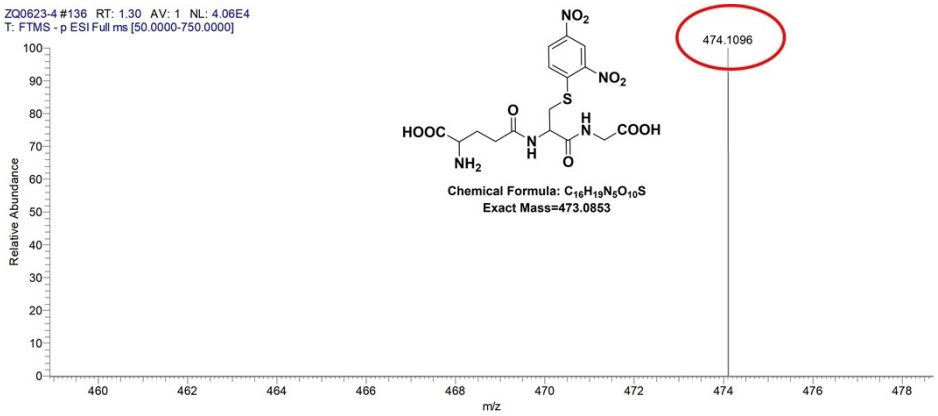
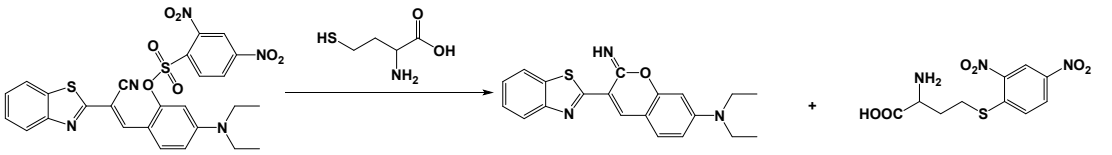




BTDA-RSS+Hcy



Chemical Formula: $C_{26}H_{21}N_5O_7S_2$
Exact Mass=579.0882

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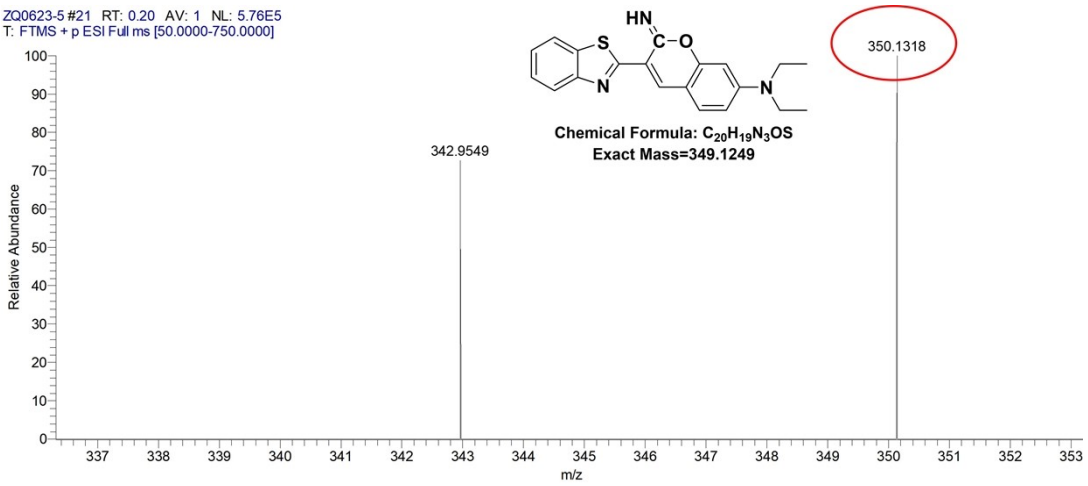
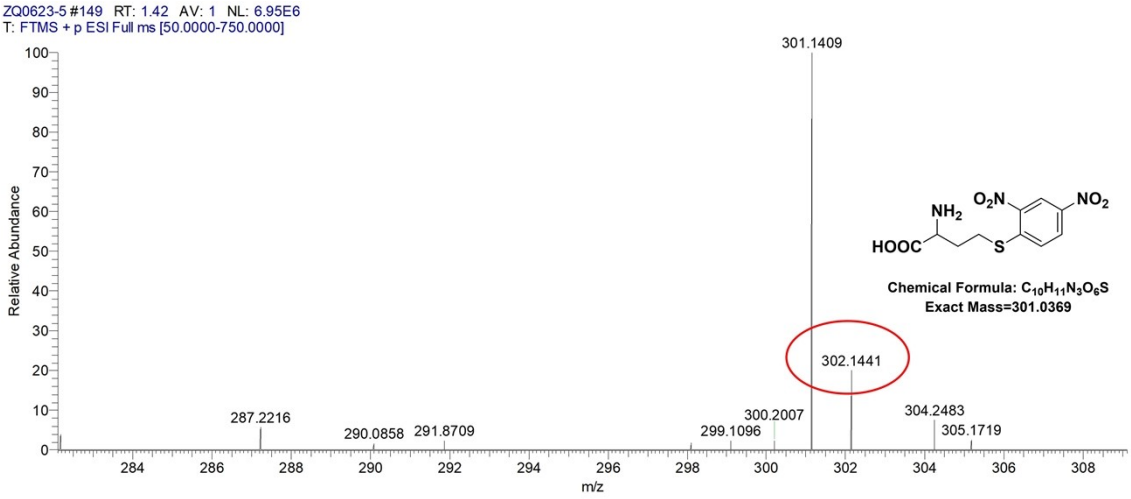
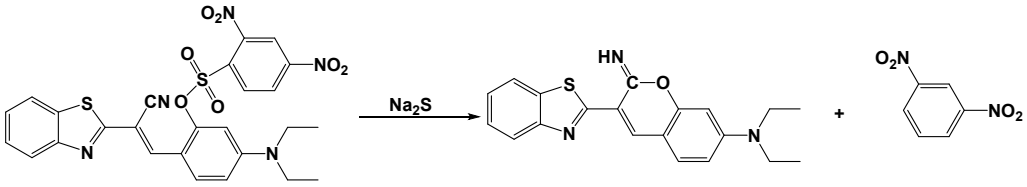




BTDA-RSS+Na_2S



Chemical Formula: $C_{26}H_{21}N_5O_7S_2$
Exact Mass=579.0882

Chemical Formula: $C_{20}H_{19}N_3OS$
Exact Mass=349.1249

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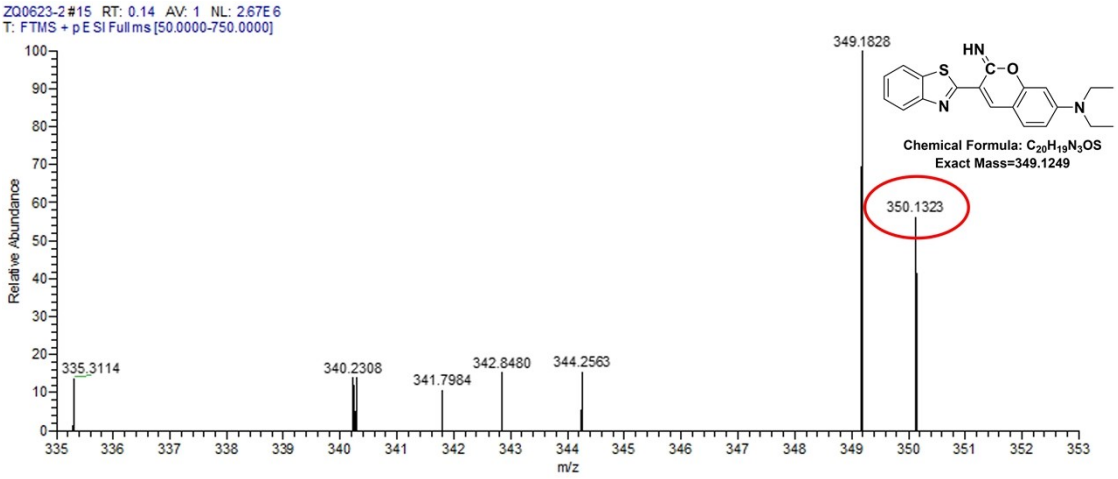


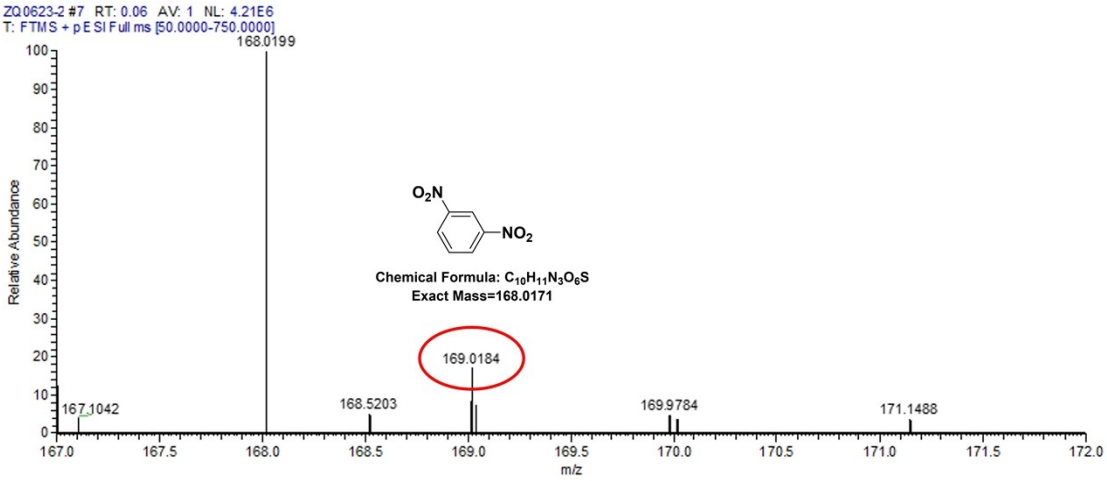


Fig. S5 HRMS analysis of **BTDA-RSS, BTDA, BTDA-RSS**+Cys, **BTDA-RSS**+GSH, **BTDA-RSS**+Hcy, **BTDA-RSS**+Na_2S and the corresponding by-products, respectively.

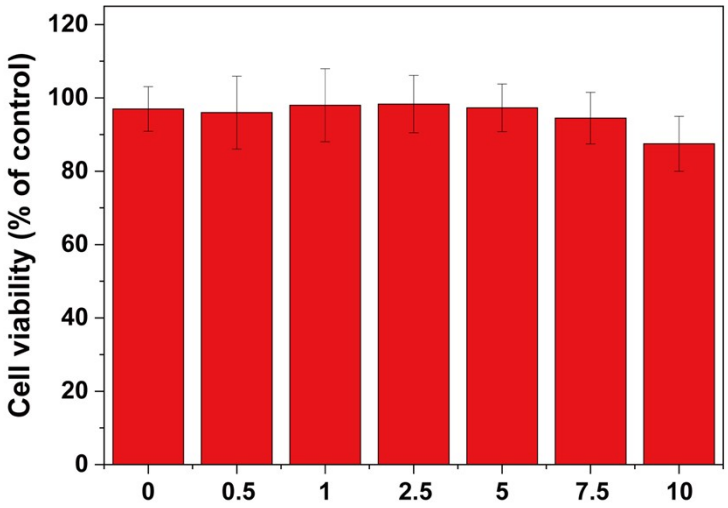
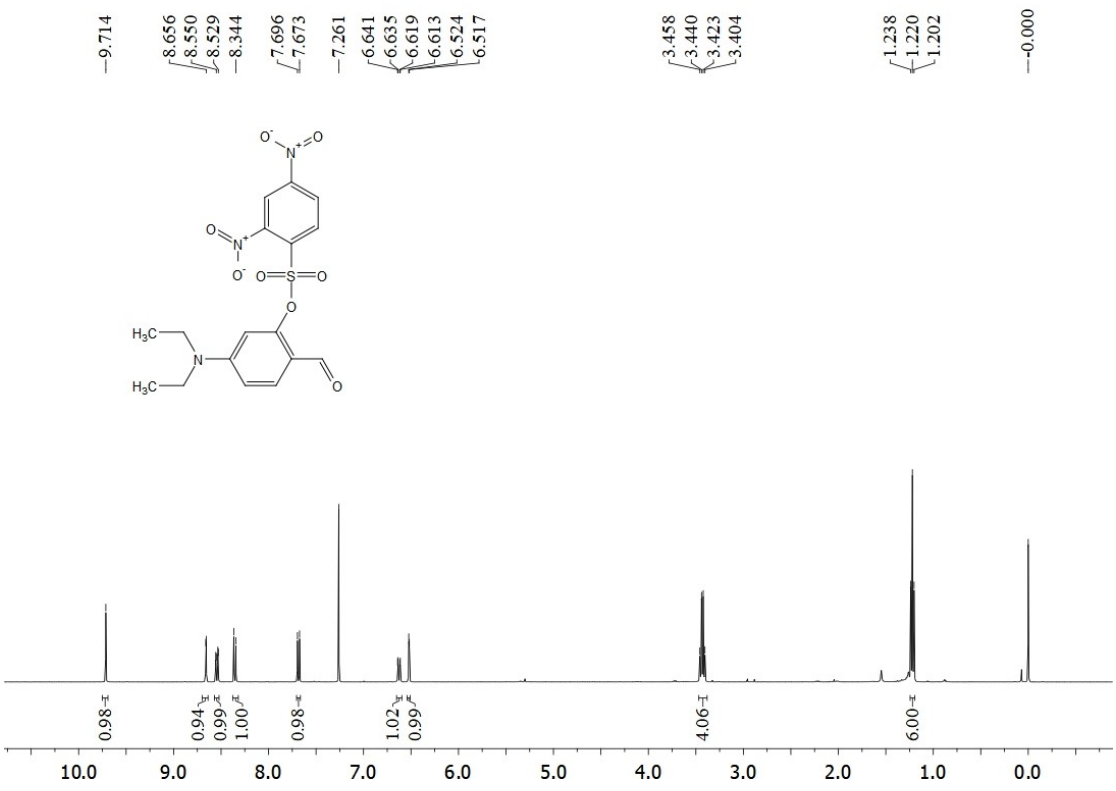
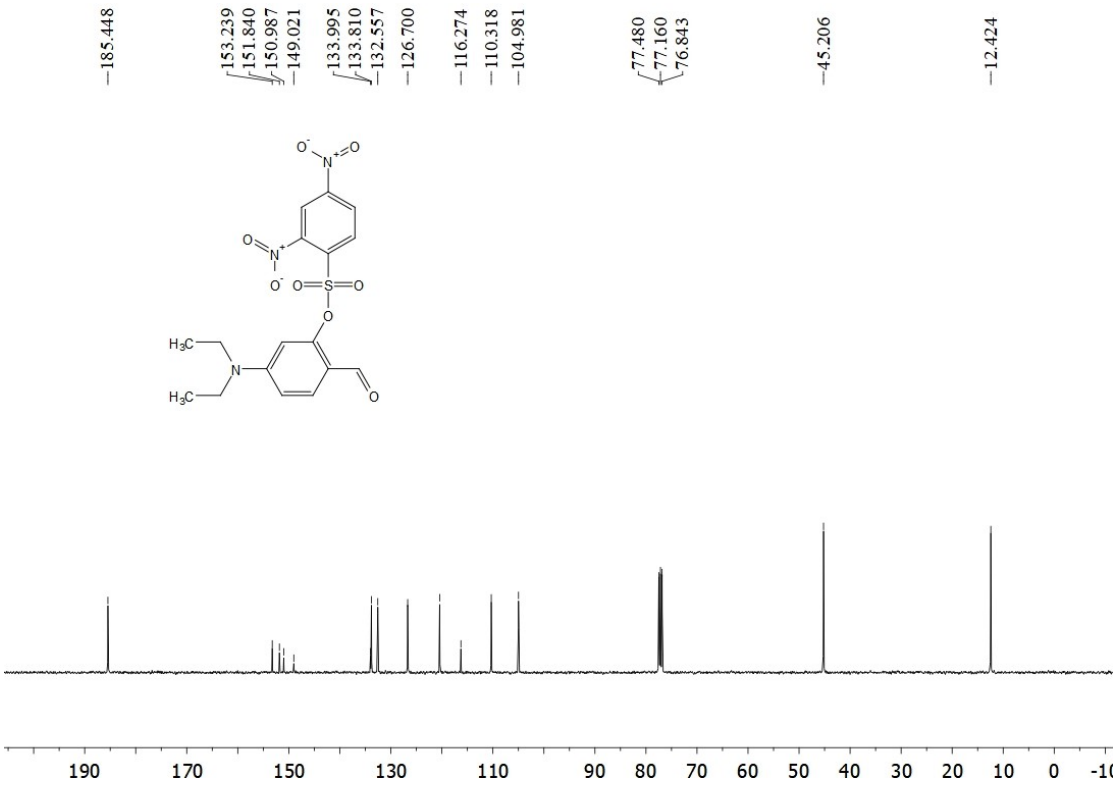
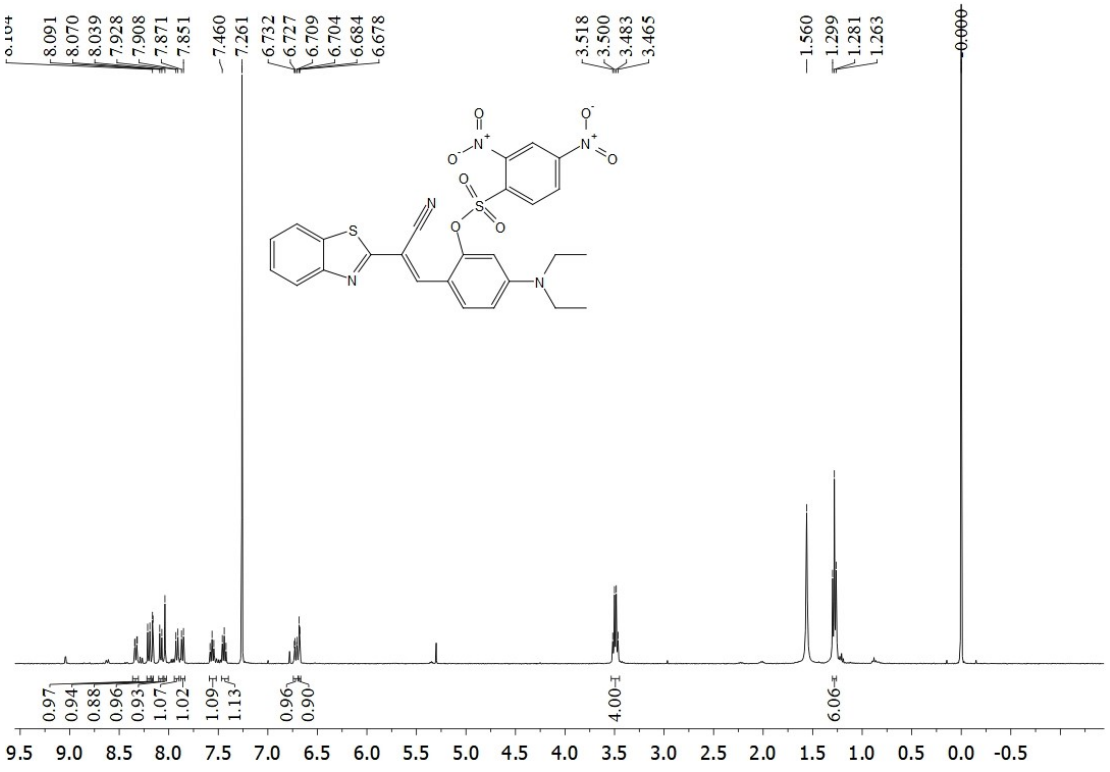


Fig. S6 Cell viability of HeLa cells treated with different concentration of **BTDA-RSS** (0, 0.5, 1, 2.5, 5, 7.5, and 10 μM).

^{1}H NMR and ^{13}C NMR spectra analysis of compound 3, BTDA-RSS and BTDA



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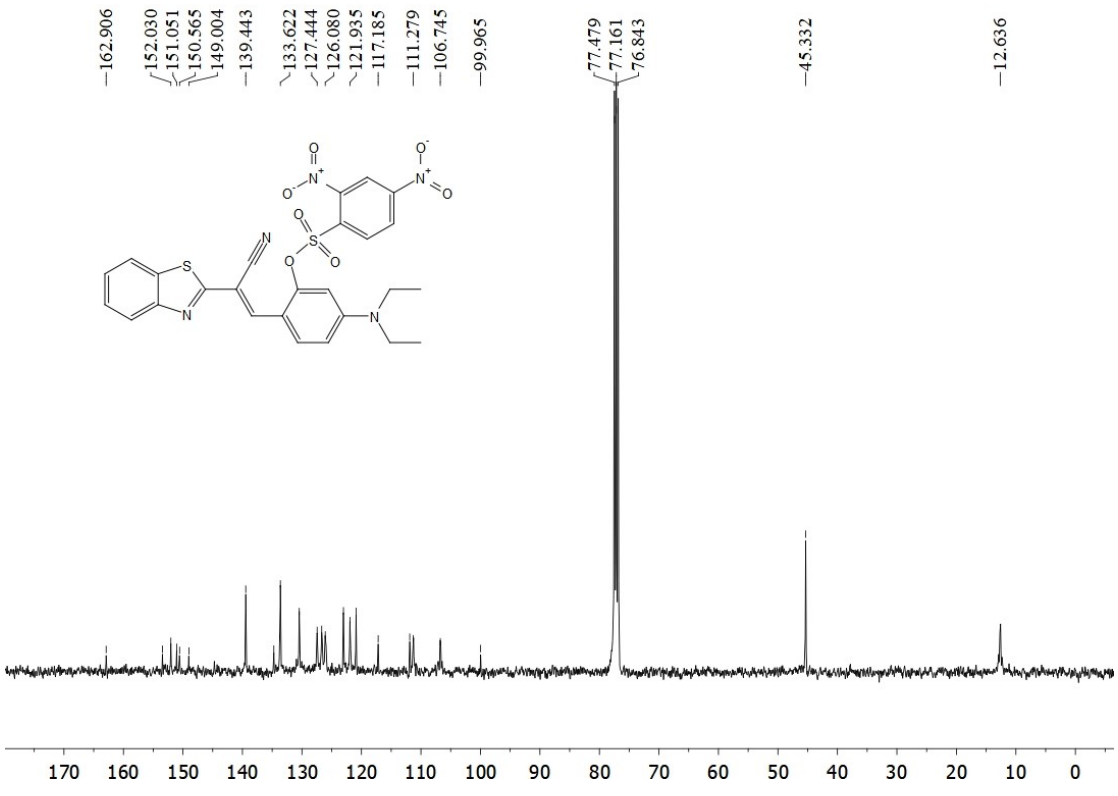
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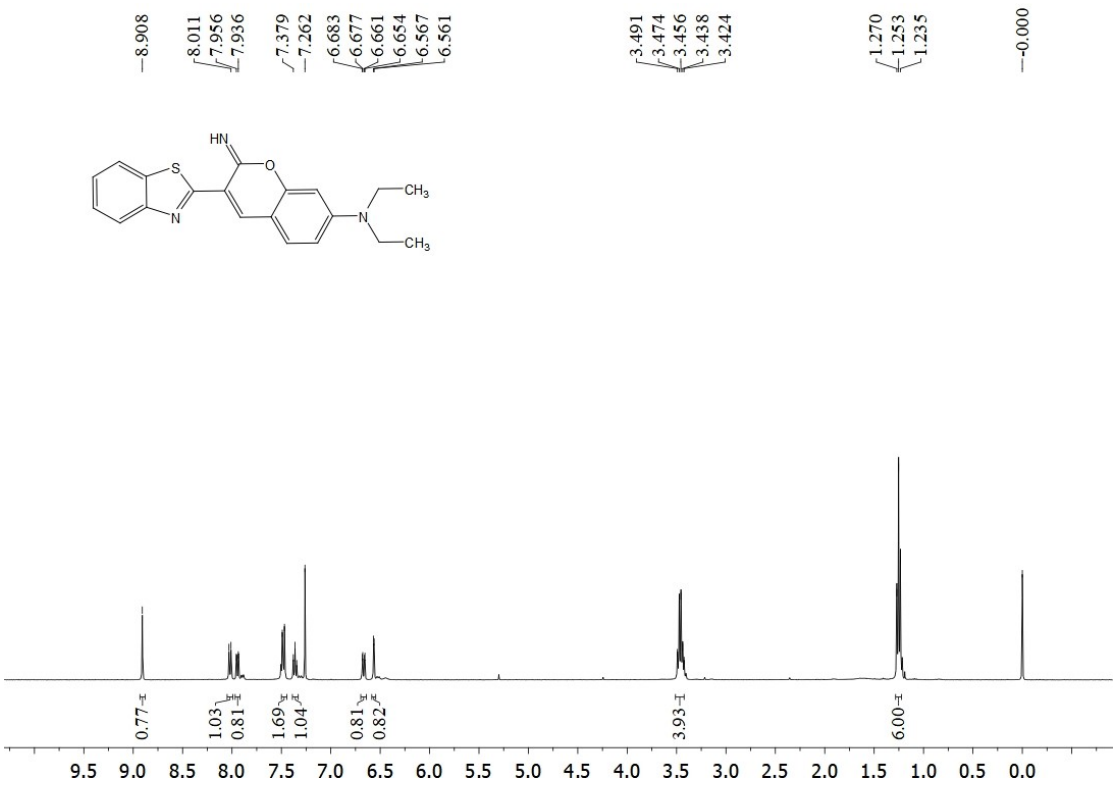
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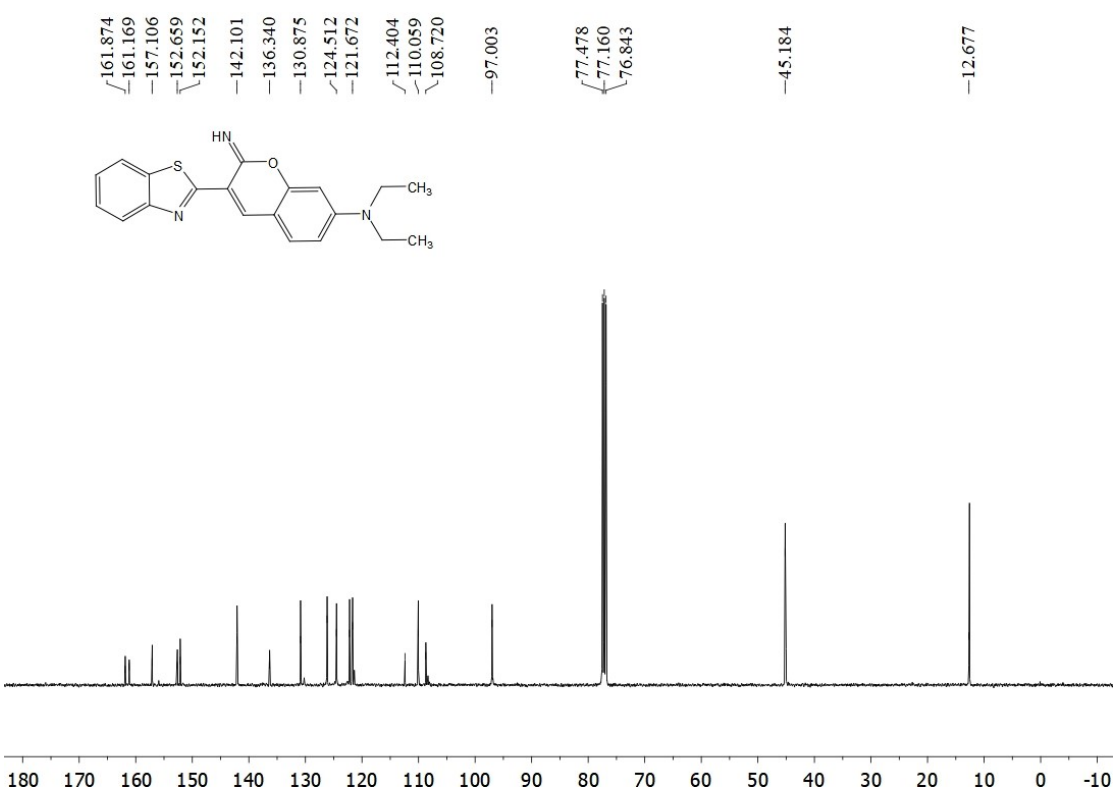
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124.512
121.672
112.404
110.059
108.720
97.003
77.478
77.160
76.843
45.184
12.677
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180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10